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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1975 through June 30, 1976

Part VI

DIVISION OF CANCER TREATMENT

United States. NATIONAL CANCER INSTITUTE

ANNUAL REPORT of program activities

July 1, 1975 through June 30, 1976

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ANNUAL REPORT

DIVISION OF CANCER TREATMENT

Vincent T. DeVita, Jr., M.D.
Director

The Division of Cancer Treatment is responsible for the development of new treatments for the control and cure of cancer. It is the main component of the National Cancer Program responsible for coordinating cancer treatment within the various elements of the National Cancer Institutes, the country and the world. The Division has five major programs, each with a separate and distinct responsibility. These programs encompass the identification of new materials or methods for the treatment of cancer at the very earliest level in rodents; their evaluation for effectiveness in standard tumor systems; the gathering of information on toxicology in preparation of IND's for submission to the Food and Drug Administration for approval to initiate clinical trials in people; the conduct of clinical trials at the earliest level (Phase I); the conduct of disease specific trials (Phase II); and finally, if therapeutic efficacy is demonstrated, comparative clinical trials against what we now consider standard treatments of cancer (Phase III). In the past year, the Division has assumed responsibility for combining into its programs not only drug and immunotherapy but surgery and radiotherapy.

The various aspects of the program for these responsibilities are as follows. The Drug Research and Development Program is responsible for the identification, work-up and development of new treatments. The program this year has taken a major change. Initial screening for the identification of activity of new drugs has been changed from the old L1210 system to a more sensitive P388 system backed up by a panel of tumors. The panel was specifically designed to ask questions in regard to the validity of screening and predicting activity of unknown chemicals in specific disease types in humans with cancer. The screen was implemented for the first time this year. In addition to the reorganization of the screening program, major reorganization or changes took place within the Division this year to further streamline its capacity to perform its mission. A new Laboratory of Medicinal Chemistry and Biology was created to give the intramural laboratory program of the DR&D Program the capacity to identify, synthesize and modify compounds within the intramural program. This laboratory is headed by Dr. David Johns, formerly of the Experimental Therapeutics Program. The Drug Development Branch, formerly responsible for the entire identification, synthesis, and provision of newly identified chemicals has been divided into three major areas. A Natural Products Branch headed by Dr. John Douros, a Synthetic Chemical Branch headed by Dr. Harry Wood and all pharmaceutical development responsibilities have been separated out into a single branch under the leadership of Mr. Paul Davignon.

Parallel to the DR&D Branch, the Experimental Therapeutics Program headed by Dr. Vincent T. Oliverio is responsible for identifying the toxicology of these new compounds in preparation for their entrance into clinical trials. This is handled under the Toxicology Branch of the National Cancer Institute. The Experimental Therapeutics Program is also responsible for identifying the

interaction of new and old chemicals at the cellular or molecular level to help elucidate the mechanism of action as well as determine the pharmacology of interesting compounds early in their investigation.

In the past year, the Drug Research and Development Program and the Experimental Therapeutics Program have taken on the added responsibility of identifying and developing analogs of radiosensitizers which includes the use of radiotherapy and radiosensitizers not only in the Drug Development Program but in the toxicology aspects of the program. This aspect of radiotherapy has formerly been done on an informal basis.

The flow from the Drug Research & Development Program and the Experimental Therapeutics Program is directly into the Cancer Therapy Evaluation Program headed by Dr. Franco Muggia, the Associate Director. This Program is responsible for the initiation and monitoring of all Phase I clinical trials conducted under the auspices of the Division. It is responsible through contract for Phase II clinical trials and in some instances for disease specific Phase III clinical trials such as those in gastrointestinal cancer conducted by the Division's Gastrointestinal Cancer Study Group. In addition, the clinical Cooperative Group program is in this area and a major responsibility of the staff of this program is to monitor the progress of all clinical trials being conducted under the auspices of the National Cancer Institute in the United States, and in the world. In addition, they serve as suppliers of chemotherapeutic agents to investigators throughout the world. They monitor these trials to provide data for Food and Drug Administration in regard to patient safety.

Two other programs within the Division of Cancer Treatment are primarily concerned with conducting laboratory research and clinical trials in specific problems in cancer of interest to investigators at the NIH.

One is the Baltimore Cancer Research Center. The Baltimore Cancer Research Center has both a clinical and laboratory capacity. The major program in the management of acute leukemia in adults is located at the Baltimore Cancer Research Center, and much work is going on in the pharmacology of the anthracycline antibiotics in this area. The two are inter-related since the disease acute myelocytic leukemia is effectively treated with anthracycline antibiotics. The oncology program of the Baltimore Cancer Research Center has recently been reorganized and divided into the Clinical Oncology Branch, the Laboratory of Molecular Biology and the Laboratory of Clinical Biochemistry. The Program is headed by Dr. Michael D. Walker, who is a neurosurgeon, and one of the responsibilities of the Program is to monitor the nationwide Brain Cancer Task Force exploring the efficacy of the use of combined modalities of treatment in brain cancer.

The remaining program, the Clinical Oncology Program headed by an Associate Director and the Clinical Director of the National Cancer Institute, consists of five intramural clinical branches; Surgery, Radiation therapy, Medicine, Pediatrics, and an additional Medical Oncology Branch located at the Veterans Administration Hospital in Washington, D.C. Each of these Branches identify specific disease areas to focus their attention on. In conjunction with therapeutic trials in these specific disease areas, investigators in the

laboratory explore the mechanism of disease and the biochemistry of cancer in carefully controlled clinical situations. It is here that the supportive care programs in the white blood cell procurement and transfusion, platelet physiology and sterile environments have been developed and continue to be explored.

All in all, the Division of Cancer Treatment represents the complete array of basic research in areas related to developmental therapeutics, all the steps intervening between the identification of basic leads up to clinical trials and the inclusion of clinical trials to improve the treatment of patients with cancer with the existing technologies, and to develop new technologies for the future.

Division of Cancer Treatment Stratification

Code	Description
PDD	Preclinical Drug Development Program -
1XX	Stage I - Acquisition of Materials
2A1	Stage II - Basic Screen - Determination of anti-tumor activity of new agents.
2B1	- Develop acceptable experimental formulation.
2B2	- Verification Screen - Detailed evaluation of new agents -- dose, route, and schedule dependency.
2B3	- Procurement of sufficient amounts of new agent for preclinical studies.
3X1	Stage III - Toxicology and pharmacology in large animals.
3X2	- Production and formulation for clinical trials.
PBR	Preclinical Basic Research -
PBS	Biological Studies - Includes biochemistry, blood products, cell biology, cell kinetics, nutrition, immunobiology, markers, molecular biology, radiobiology, transfusion research, data processing
PTS	Treatment Studies - Independent treatments, combined modality therapy
CTR	Clinical Trials Research -
CT1	Phase I Clinical Trials* - initial clinical evaluation of new drugs, clinical pharmacology.
CT2	Phase II Clinical Trials* - allocation for specific disease-oriented resources to study whatever chemotherapy (single agents or combinations) or combined modality regimens have highest priority for initial efficacy evaluation.
CT3	Phase III Clinical Trials* - allocation for specific disease-oriented resources to study whatever chemotherapy or combined modality regimens have highest priority for efficacy evaluation in a controlled clinical setting.
CT4	Phase IV Clinical Trials* - allocation for specific disease-oriented resources to evaluate the combined modality approach to the initial therapeutic attack on local or regional disease in an attempt to increase the number of patients with a long disease-free period.

CT5 Statistics, data processing, other clinical trials research.

CSR Clinical Trials Supportive Research - Includes Special Pharm./Tox., cell kinetics, markers, blood products, transfusion research, protected environment, hyperthemia, nutrition, statistics, data processing.

MGT Program Management - Includes administration, dissemination of information to medical and scientific community.

*Supportive care used as ancillary therapy should be prorated among the phases of clinical trials using such resources.

TABLE I

	ANNUAL LEVEL *	PERCENT
PDD PRECLINICAL DRUG DEVELOPMENT PROGRAM	40,502,317	60.97
STAGE I	12,381,726	18.64
1XX ACQUISITION OF MATERIALS		
STAGE IIA	8,481,009	12.77
2A1 DETERMINATION OF ANTI-TUMOR ACTIVITY		
STAGE IIB	11,320,674	17.04
2B1 EXPERIMENTAL FORMULATION DEVELOPMENT	280,578	0.42
2B2 DETAILED EVALUATION OF NEW AGENTS	7,226,397	10.88
2B3 PROCURE. OF AGENTS FOR PRECLIN. STUDIES	3,813,699	5.74
STAGE III	8,318,908	12.52
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	4,412,609	6.64
3X2 PROD. & FORM. FOR CLINICAL TRIALS	3,906,299	5.88
PBR PRECLINICAL BASIC RESEARCH	4,058,271	6.11
PBS BIOLOGICAL STUDIES	2,766,584	4.16
PTS TREATMENT STUDIES	1,291,687	1.94
CTR CLINICAL TRIALS RESEARCH	14,169,857	21.33
CT1 PHASE I CLINICAL TRIALS	1,649,173	2.48
CT2 PHASE II CLINICAL TRIALS	3,840,299	5.78
CT3 PHASE III CLINICAL TRIALS	5,192,291	7.82
CT4 PHASE IV CLINICAL TRIALS	3,488,094	5.25
CSR CLINICAL TRIALS SUPPORTIVE RESEARCH	5,902,363	8.89
MGT PROGRAM MANAGEMENT	1,797,489	2.71
TOTAL	66,430,297	100.00

* FY-76 FUNDING REQUIREMENTS AS OF FEB. 29, 1976 FOR APPROXIMATELY 301 CONTRACTS. INCLUDED ARE 25 SCHEDULE A CONTRACTS ($4,425,685) AND 14 SCHEDULE B CONTRACTS ($2,475,000). NOT INCLUDED ARE FUNDS UTILIZED FOR DIRECT PURCHASE OF CLINICAL DRUGS (APPROXIMATELY $3,700,000).

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
STAGE I ACQUISITION OF MATERIALS:		
ABBOTT LABORATORIES	N01CM43704	87,554
AGRICULTURE, DEPARTMENT OF	Y01CM40001	389,797
ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO50004	200,003
ARS/SPRAGUE-DAWLEY	N01CM53813	162,790
ARS/SPRAGUE-DAWLEY	N01CM53846	43,028
ARTHUR D. LITTLE, INC.	N01CM67078	10,514
ARTHUR D. LITTLE, INC.	N01CM63823	32,285
ARTHUR D. LITTLE, INC.	N01CM33727	26,457
ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271	217,742
BELLAIRE ACRES	N01CM50583	41,808
BRISTOL-MYERS COMPANY	N01CM43759	223,578
BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	73,903
CHARLES RIVER BREEDING LABS.	N01CM53848	41,046
CHARLES RIVER BREEDING LABS.	N01CM50598	45,240
CHARLES RIVER BREEDING LABS.	N01CM53812	233,987
CHEMICAL ABSTRACTS SERVICE	N01CM43722	452,000

CONTRACT	#	DOLLAR LEVEL
STAGE I ACQUISITION OF MATERIALS:		
CHESTER BEATTY RESEARCH INSTITUTE	NO1CM43736	16,000
COLLABORATIVE RESEARCH, INC.	NO1CM60506	40,500
COLLABORATIVE RESEARCH, INC.	NO1CM12326	80,000
FARMITALIA	NO1CM57036	158,060
FARMITALIA	NO1CM57014	161,174
FLOW LABORATORIES	NO1CM50571	43,680
GEORGE WASHINGTON UNIVERSITY	NO1CM12206	14,328
GEORGETOWN UNIVERSITY	NO1CM43732	64,882
HARLAN INDUSTRIES	NO1CM23218	8,621
HARLAN INDUSTRIES	NO1CM50591	43,992
HAWAII, UNIVERSITY OF	NO1CM33747	84,000
HAZLETON LABORATORIES, INC.	NO1CM33708	76,394
HAZLETON LABORATORIES, INC.	NO1CM57007	1,223
HEALTH RESEARCH, INC.	NO1CM02035	30,313
IIT RESEARCH INSTITUTE	NO1CM53829	24,401
IIT RESEARCH INSTITUTE	NO1CM43755	44,920
IIT RESEARCH INSTITUTE	NO1CM63832	3,870
INSTITUT JULES BORDET	NO1CM53840	18,810

CONTRACT	#	DOLLAR LEVEL
STAGE I ACQUISITION OF MATERIALS:		
JACKSON LABORATORY	N01CM60176	26,364
JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	4,732
KANSAS, UNIVERSITY OF	N01CM53819	20,706
LABORATORY SUPPLY CO.	N01CM50577	43,992
LABORATORY SUPPLY CO.	N01CM53775	25,946
LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM53814	88,481
LITTON BIONETICS, INC.	N01CO25423	1,991,261
LITTON BIONETICS, INC.	N01CM53779	14,234
MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM33720	26,865
MASSACHUSETTS INSTITUTE OF TECHNOLO	N01CM43712	60,643
MIAMI, UNIVERSITY OF	N01CM67077	6,247
MICROBIAL CHEMISTRY RESEARCH FDN.	N01CM57009	39,600
MICROBIOLOGICAL ASSOCIATES	N01CM67069	35,160
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM43793	261,480
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM53818	28,700
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM43761	377,760
MIDWEST RESEARCH INSTITUTE	N01CM33743	300,504
MURPHY BREEDING LABS.	N01CM50579	41,702

CONTRACT	#	DOLLAR LEVEL
STAGE I ACQUISITION OF MATERIALS:		
NORTHEASTERN UNIVERSITY	N01CM53741	56,020
OHIO STATE UNIVERSITY	N01CM57015	57,894
PARKE, DAVIS AND COMPANY	N01CM43754	634,169
PENNSYLVANIA, UNIVERSITY OF	N01CM33719	117,864
PFIZER, INC.	N01CM53805	359,861
POMONA COLLEGE	N01CM33703	43,800
RHODE ISLAND, UNIVERSITY OF	N01CM43778	41,458
SIMONSEN LABORATORIES	N01CM53817	65,382
SIMONSEN LABORATORIES	N01CM50578	44,616
SIMONSEN LABORATORIES	N01CM53847	44,035
SIMONSEN LABORATORIES	N01CM40884	428
SMALL BUSINESS ADMINISTRATIC I	N01CM43719	67,653
SOUTHERN ANIMAL FARMS	N01CM53845	26,932
SOUTHERN ANIMAL FARMS	N01CM50599	42,432
SOUTHERN RESEARCH INSTITUTE	N01CM43762	527,782
SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATIO	N01CM67075	4,886
SPARTAN RESEARCH ANIMALS, INC.	N01CM50582	21,216
STANFORD RESEARCH INSTITUTE	N01CM33742	356,430

CONTRACT	#	DOLLAR LEVEL
STAGE I ACQUISITION OF MATERIALS:		
STARKS, C. P., INC.	N01CM53745	373,263
TACONIC FARMS	N01CM50597	34,320
TEXAS INBRED MICE	N01CM53815	94,655
TULANE UNIVERSITY	N01CM53815	39,101
UPJOHN COMPANY	N01CM43753	684,273
UTAH, UNIVERSITY OF	N01CM43806	157,679
VALUE ENGINEERING COMPANY	N01CM33706	125,821
VETERANS ADMINISTRATION HOSPITAL	Y01CM60102	40,925
WARF INSTITUTE, INC.	N01CM43707	111,287
WISCONSIN, UNIVERSITY OF	N01CM33733	240
WISCONSIN, UNIVERSITY OF	N01CM67076	2,813
YALE UNIVERSITY	N01CM57029	67,815
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM53824	54,296
TOTAL		10,890,623

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
STAGE II BASIC SCREEN:		
ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO50004	50,000
ARS/SPRAGUE-DAWLEY	N01CM53813	447,673
ARS/SPRAGUE-DAWLEY	N01CM53846	118,329
ARTHUR D. LITTLE, INC.	N01CM53849	98,249
ARTHUR D. LITTLE, INC.	N01CM67078	199,753
ARTHUR D. LITTLE, INC.	N01CM53765	269,839
ARTHUR D. LITTLE, INC.	N01CM33727	66,137
BELLAIRE ACRES	N01CM50583	114,972
CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM23703	45,000
CHARLES RIVER BREEDING LABS.	N01CM53848	112,879
CHARLES RIVER BREEDING LABS.	N01CM50598	124,410
CHARLES RIVER BREEDING LABS.	N01CM53812	643,464
FLOW LABORATORIES	N01CM50571	120,120
HARLAN INDUSTRIES	N01CM23218	23,708
HARLAN INDUSTRIES	N01CM50591	120,978
HAZLETON LABORATORIES, INC.	N01CM57007	4,895

CONTRACT	#	DOLLAR LEVEL
STAGE II BASIC SCREEN:		
HEALTH RESEARCH, INC.	N01CM33726	14,339
HEALTH RESEARCH, INC.	N01CM02035	83,360
IIT RESEARCH INSTITUTE	N01CM53829	67,104
IIT RESEARCH INSTITUTE	N01CM43755	145,989
IIT RESEARCH INSTITUTE	N01CM63832	73,535
INSTITUT JULES BORDET	N01CM53840	15,675
JACKSON LABORATORY	N01CM60176	72,501
JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	9,461
KANSAS, UNIVERSITY OF	N01CM53819	56,942
LABORATORY SUPPLY CO.	N01CM50577	120,978
LABORATORY SUPPLY CO.	N01CM53775	71,352
LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM53814	243,322
LITTON BIONETICS, INC.	N01CM33741	23,100
LITTON BIONETICS, INC.	N01CO25423	497,815
LITTON BIONETICS, INC.	N01CM53778	39,145
MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM33720	125,370
MIAMI, UNIVERSITY OF	N01CM67077	118,706
MICHIGAN DEPARTMENT OF PUBLIC HEALTH	N01CM33702	54,166

CONTRACT	#	DOLLAR LEVEL
STAGE II BASIC SCREEN:		
MICROBIAL CHEMISTRY RESEARCH FDN.	N01CM57009	26,400
MICROBIOLOGICAL ASSOCIATES	N01CM67069	96,690
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM53818	78,925
MIDWEST RESEARCH INSTITUTE	N01CM33743	22,538
MURPHY BREEDING LABS.	N01CM50579	114,680
PARKE, DAVIS AND COMPANY	N01CM43754	111,912
SIMONSEN LABORATORIES	N01CM53817	179,802
SIMONSEN LABORATORIES	N01CM50578	122,694
SIMONSEN LABORATORIES	N01CM53847	121,096
SIMONSEN LABORATORIES	N01CM40884	1,178
SOUTHERN ANIMAL FARMS	N01CM53845	74,063
SOUTHERN ANIMAL FARMS	N01CM50599	116,688
SOUTHERN RESEARCH INSTITUTE	N01CM43756	758,400
SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATIO	N01CM67075	92,835
SPARTAN RESEARCH ANIMALS, INC.	N01CM50582	58,344
STANFORD RESEARCH INSTITUTE	N01CM33742	19,010
TACONIC FARMS	N01CM50597	94,380
TEXAS INBRED MICE	N01CM53816	260,301

CONTRACT	#	DOLLAR LEVEL
STAGE II BASIC SCREEN:		
TULANE UNIVERSITY	N01CM53815	107,527
UPJOHN COMPANY	N01CM43753	204,082
UTAH, UNIVERSITY OF	N01CM43806	22,526
VALUE ENGINEERING COMPANY	N01CM33706	521,260
WISCONSIN, UNIVERSITY OF	N01CM33733	445
WISCONSIN, UNIVERSITY OF	N01CM67076	53,449
YALE UNIVERSITY	N01CM57029	22,606
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM53824	26,743
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM33711	6,300
TOTAL		7,708,140

ANALYSIS OF CONTRACTS BY ACTIVITY

FOR FISCAL YEAR 1976
AS OF 02/29/76

ACTIVITY	CONTRACT #	DOLLAR LEVEL
STAGE II BIOLOGICAL RESEARCH PROGRAMS:		
ARIZONA, UNIVERSITY OF	N01CM33713	24,325
ARTHUR D. LITTLE, INC.	N01CM53849	184,216
ARTHUR D. LITTLE, INC.	N01CM63823	181,970
ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271	27,218
BRIGHAM YOUNG UNIVERSITY	N01CM43790	24,305
HARVARD UNIVERSITY	N01CM53825	40,708
HAZLETON LABORATORIES, INC.	N01CM43702	408,580
HAZLETON LABORATORIES, INC.	N01CM33708	114,591
HAZLETON LABORATORIES, INC.	N01CM23701	147,614
HEALTH RESEARCH, INC.	N01CM57039	38,006
HEBREW UNIVERSITY	N01CM12127	60,000
JOHNS HOPKINS UNIVERSITY	N01CM43718	41,000
LITTON BIONETICS, INC.	N01CM22062	83,656
LITTON BIONETICS, INC.	N01CM12340	705,209
LITTON BIONETICS, INC.	N01CP33211	25,000
MIAMI, UNIVERSITY OF	N01CM57041	38,216
NAVAL WEAPONS CENTER	Y01CM50101	7,435

CONTRACT	#	DOLLAR LEVEL
STAGE II BIOLOGICAL RESEARCH PROGRAMS:		
RES. FDN. OF STATE UNIVERSITY OF NEW YORK	N01CM53766	56,461
SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N01CM53820	59,177
SMALL BUSINESS ADMINISTRATION	N01CM43719	28,395
SOUTHERN RESEARCH INSTITUTE	N01CM43756	118,500
WISCONSIN, UNIVERSITY OF	N01CM12275	95,000
TOTAL		2,510,182

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY — FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
STAGE II FORMULATION:		
BEN VENUE LABORATORIES, INC.	N01CM53811	63,103
IOWA, UNIVERSITY OF	N01CM43743	32,469
KANSAS, UNIVERSITY OF	N01CM23217	100,000
PHILIPS ROXANE LABORATORIES, INC.	N01CM12139	8,000
TENNESSEE, UNIVERSITY OF	N01CM02084	5,000
UPJOHN COMPANY	N01CM43753	12,005
TOTAL		220,577

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
STAGE II VERIFICATION SCREEN:		
ALLEGHENY GENERAL HOSPITAL	N01CM43730	67,288
ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO50004	125,002
ARS/SPRAGUE-DAWLEY	N01CM53813	203,488
ARS/SPRAGUE-DAWLEY	N01CM53846	53,786
ARTHUR D. LITTLE, INC.	N01CM67047	118,916
ARTHUR D. LITTLE, INC.	N01CM53765	154,193
ARTHUR D. LITTLE, INC.	N01CM33727	66,137
ATOMIC ENERGY COMMISSION	Y01CM10056	25,500
BAYLOR COLLEGE OF MEDICINE	N01CM57018	34,000
BELLAIRE ACRES	N01CM50593	52,260
BRIGHAM YOUNG UNIVERSITY	N01CM43790	56,714
CALIFORNIA, UNIVERSITY OF	N01CM43791	46,405
CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM23703	247,500
CHARLES RIVER BREEDING LABS.	N01CM53848	51,309
CHARLES RIVER BREEDING LABS.	N01CM50598	56,550
CHARLES RIVER BREEDING LABS.	N01CM53812	292,484

CONTRACT	#	DOLLAR LEVEL
STAGE II VERIFICATION SCREEN:		
CHESTER BEATTY RESEARCH INSTITUTE	N01CM43736	16,000
FLOW LABORATORIES	N01CM50571	54,600
HARLAN INDUSTRIES	N01CM23218	10,776
HARLAN INDUSTRIES	N01CM50591	54,990
HARVARD UNIVERSITY	N01CM53825	17,446
HAZLETON LABORATORIES, INC.	N01CM33708	38,197
HAZLETON LABORATORIES, INC.	N01CM57007	1,795
HEALTH RESEARCH, INC.	N01CM57039	57,010
HEALTH RESEARCH, INC.	N01CM02035	37,891
HEALTH RESEARCH, INC.	N01CM43785	57,210
IIT RESEARCH INSTITUTE	N01CM53829	30,502
IIT RESEARCH INSTITUTE	N01CM43755	33,690
JACKSON LABORATORY	N01CM60176	32,955
JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	9,461
KANSAS, UNIVERSITY OF	N01CM53819	25,883
LABORATORY SUPPLY CO.	N01CM50577	54,990
LABORATORY SUPPLY CO.	N01CM53775	32,433
LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM53814	110,601

CONTRACT	#	DOLLAR LEVEL
STAGE II VERIFICATION SCREEN:		
LITTON BIONETICS, INC.	N01CM33741	53,900
LITTON BIONETICS, INC.	N01CO25423	1,244,538
LITTON BIONETICS, INC.	N01CM53778	17,793
MADRID, UNIVERSITY OF	N01CM53792	30,016
MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM33720	26,865
MASON RESEARCH INSTITUTE	N01CM57030	155,488
MIAMI, UNIVERSITY OF	N01CM57041	57,324
MICHIGAN CANCER FOUNDATION	N01CM53789	53,671
MICROBIOLOGICAL ASSOCIATES	N01CM67069	43,950
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM53819	35,875
MINNESOTA, UNIVERSITY OF	N01CM43788	70,801
MURPHY BREEDING LABS.	N01CM50579	52,127
PENNSYLVANIA, UNIVERSITY OF	N01CM53830	22,656
RES. FDN. OF STATE UNIVERSITY OF NEW YORK	N01CM53765	84,692
SIMONSEN LABORATORIES	N01CM53817	81,728
SIMONSEN LABORATORIES	N01CM50578	55,770
SIMONSEN LABORATORIES	N01CM53847	55,044
SIMONSEN LABORATORIES	N01CM40884	535

CONTRACT	#	DOLLAR LEVEL
STAGE II VERIFICATION SCREEN:		
SOUTHERN ANIMAL FARMS	N01CM53845	33,665
SOUTHERN ANIMAL FARMS	N01CM50599	53,040
SOUTHERN RESEARCH INSTITUTE	N01CM43756	1,137,600
SOUTHERN RESEARCH INSTITUTE	N01CM43784	400,804
SPARTAN RESEARCH ANIMALS, INC.	N01CM50582	26,520
TACONIC FARMS	N01CM50597	42,900
TEXAS INBRED MICE	N01CM53816	118,319
TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM43786	85,550
TEXAS, UNIVERSITY OF	N01CM53767	66,773
TULANE UNIVERSITY	N01CM53815	48,876
UPJOHN COMPANY	N01CM43753	120,048
VALUE ENGINEERING COMPANY	N01CM33706	179,745
WASHINGTON UNIVERSITY SCHOOL OF MEDICINE	N01CM43731	89,794
YESHIVA UNIVERSITY	N01CM43787	91,632
TOTAL		6,966,001

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
STAGE II PROCUREMENT OF PRECLIN. MAT'L:		
AGRICULTURE, DEPARTMENT OF	Y01CM40001	43,311
ALDRICH CHEMICAL COMPANY, INC.	N01CM23706	134,270
ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO50004	41,667
ASH STEVENS, INC.	N01CM12314	2,112
ASH STEVENS, INC.	N01CM57012	160,657
ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271	27,218
BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	4,619
COLLABORATIVE RESEARCH, INC.	N01CM60506	4,500
CORDOVA CHEMICAL COMPANY	N01CM57031	60,853
DOW CHEMICAL COMPANY	N01CM53768	60,752
FARMITALIA	N01CM57036	17,562
FARMITALIA	N01CM57014	17,908
LITTON BIONETICS, INC.	N01CM22062	195,197
LITTON BIONETICS, INC.	N01CM12340	705,210
LITTON BIONETICS, INC.	N01CP33211	25,000
LITTON BIONETICS, INC.	N01CO25423	414,846
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM43793	130,740

CONTRACT	#	DOLLAR LEVEL
STAGE II PROCUREMENT OF PRECLIN. MAT'L:		
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM43761	19,882
MIDWEST RESEARCH INSTITUTE	N01CM33722	43,592
MIDWEST RESEARCH INSTITUTE	N01CM33743	52,588
MONSANTO RESEARCH CORPORATION	N01CM33709	120,888
NORTHEASTERN UNIVERSITY	N01CM53741	2,948
OHIO STATE UNIVERSITY	N01CM57015	6,433
PARKE, DAVIS AND COMPANY	N01CM23708	202,822
PHARM-ECO	N01CM23707	54,182
RHODE ISLAND, UNIVERSITY OF	N01CM43778	4,606
SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N01CM53820	19,726
SOUTHERN RESEARCH INSTITUTE	N01CM43762	131,946
STANFORD RESEARCH INSTITUTE	N01CM33723	69,158
STANFORD RESEARCH INSTITUTE	N01CM33742	99,800
STARKS ASSOCIATES, INC.	N01CM23203	196,288
STARKS ASSOCIATES, INC.	N01CM53769	125,317
UTAH, UNIVERSITY OF	N01CM43806	45,051
VETERANS ADMINISTRATION HOSPITAL	Y01CM60102	4,547
TOTAL		3,246,196

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
STAGE III PHARMACOLOGY/TOXICOLOGY:		
ARTHUR D. LITTLE, INC.	N01CM53849	945,643
BATTELLE MEMORIAL INSTITUTE	N01CM43746	1,500,000
BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223	13,857
GEORGE WASHINGTON UNIVERSITY	N01CM12206	57,313
HAZLETON LABORATORIES, INC.	N01CM43705	32,010
HAZLETON LABORATORIES, INC.	N01CM33708	152,788
HAZLETON RESEARCH ANIMALS, INC.	N01CM60125	40,500
HAZLETON RESEARCH ANIMALS, INC.	N01CM41150	1,324
HEALTH RESEARCH, INC.	N01CM33726	3,585
LABORATORY RESEARCH ENTERPRISES, INC.	N01CM60124	39,750
MARSHALL RESEARCH ANIMALS, INC.	N01CM60123	42,000
OHIO STATE UNIVERSITY	N01CM53828	6,000
OREGON STATE UNIVERSITY	N01CM23201	93,840
PUBLIC HEALTH SERVICE	Y01CM40003	87,383
SOUTHERN RESEARCH INSTITUTE	N01CM43780	457,008
SOUTHERN RESEARCH INSTITUTE	N01CM57000	252,381
TEXAS, UNIV. OF-M.D. ANDERSON	N01CM53773	291,638

CONTRACT	#	DOLLAR LEVEL
STAGE III PHARMACOLOGY/TOXICOLOGY:		
UPJOHN COMPANY	N01CM43753	180,072
UTAH, UNIVERSITY OF	N01CM53827	62,362
VETERANS ADMINISTRATION	Y01CM60103	554
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM33711	7,350
TOTAL		4,267,358

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
STAGE III PRECLIN. COMBINED TREATMENT:		
ALLEGHENY GENERAL HOSPITAL	N01CM43730	22,430
ARTHUR D. LITTLE, INC.	N01CM53765	346,935
ARTHUR D. LITTLE, INC.	N01CM33727	6,613
CALIFORNIA, UNIVERSITY OF	N01CM43791	46,406
CATHOLIC MEDICAL CTR OF BROOKLYN AND QUEENS	N01CM23703	157,500
HAZLETON LABORATORIES, INC.	N01CM57007	245
MARIO NEGRI INST OF PHARMACOLOGICAL RESEARCH	N01CM53826	86,436
RADIOBIOLOGICAL INSTITUTE	N01CM53763	67,111
SOUTHERN RESEARCH INSTITUTE	N01CM43756	355,500
SOUTHERN RESEARCH INSTITUTE	N01CM57000	108,164
VALUE ENGINEERING COMPANY	N01CM33706	71,898
WASHINGTON UNIVERSITY SCHOOL OF MEDICINE	N01CM43731	22,449
TOTAL		1,291,687

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
STAGE III PROD./FORM. FOR CLIN. TRIALS:		
ALDRICH CHEMICAL COMPANY, INC.	N01CM23706	57,545
ASH STEVENS, INC.	N01CM12314	704
ASH STEVENS, INC.	N01CM57012	53,552
BEN VENUE LABORATORIES, INC.	N01CM53811	567,925
CORDOVA CHEMICAL COMPANY	N01CM57031	182,560
DOW CHEMICAL COMPANY	N01CM53768	182,256
IOWA, UNIVERSITY OF	N01CM43743	129,879
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM43793	43,580
MIDWEST RESEARCH INSTITUTE	N01CM33722	174,366
MONSANTO RESEARCH CORPORATION	N01CM33709	1,087,991
PARKE, DAVIS AND COMPANY	N01CM23708	202,824
PHARM-ECO	N01CM23707	126,425
PHILIPS ROXANE LABORATORIES, INC.	N01CM12139	32,000
STANFORD RESEARCH INSTITUTE	N01CM33723	276,631
STAPKS ASSOCIATES, INC.	N01CM23203	196,288
STAPKS ASSOCIATES, INC.	N01CM53769	41,773
TENNESSEE, UNIVERSITY OF	N01CM02084	15,000
TOTAL		3,371,299

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
PROGRAM MANAGEMENT:		
ABBOTT LABORATORIES	N01CM43704	58,368
ARTHUR D. LITTLE, INC.	N01CM63823	44,025
BOWMAN GRAY SCHOOL OF MEDICINE	N01CM67054	3,856
CALIFORNIA, UNIVERSITY OF	N01CM53752	12,147
CALIFORNIA, UNIVERSITY OF	N01CM67055	8,696
CLINICA NEUROCHIRURGICA DELL UNIVERSI	N01CM67056	2,100
CONTROL DATA CORPORATION	N01CM33739	5,000
DOT SYSTEMS, INC.	N01CM53774	150,000
DUKE UNIVERSITY	N01CM67010	10,694
GEORGETOWN UNIVERSITY	N01CM12123	22,050
GEORGETOWN UNIVERSITY	N01CM33745	895,883
HAZLETON LABORATORIES, INC.	N01CM43705	990
HAZLETON LABORATORIES, INC.	N01CM33705	28,718
INDIANA UNIVERSITY FOUNDATION	N01CM67057	5,192
INSTITUT JULES BORDET	N01CM53840	28,215
JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	9,462
KENTUCKY, UNIVERSITY OF	N01CM67058	6,992

CONTRACT	#	DOLLAR LEVEL
PROGRAM MANAGEMENT:		
MARYLAND, UNIVERSITY OF	N01CM43748	252,000
MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	12,998
MONTEFIORE HOSPITAL	N01CM67061	5,627
NATIONAL ACADEMY OF SCIENCES	N01CM57013	23,000
NATIONAL ACADEMY OF SCIENCES	N01CM53850	20,000
NEW YORK UNIVERSITY MEDICAL CENTER	N01CM71174	383
NEW YORK UNIVERSITY MEDICAL CENTER	N01CM67059	9,161
OHIO STATE UNIVERSITY RESEARCH FOUNDATI	N01CM67060	4,677
PUBLIC HEALTH SERVICE	Y01CM40003	14,564
ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	11,283
TENNESSEE, UNIVERSITY OF	N01CM57021	9,972
VALUE ENGINEERING COMPANY	N01CM53821	105,978
VERMONT, UNIVERSITY OF	N01CM33716	6,768
TOTAL		1,768,799

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
PHASE I CLINICAL TRIALS:		
ARTHUR D. LITTLE, INC.	N01CM63823	35,220
MARYLAND, UNIVERSITY OF	N01CM43748	432,000
MAYO FOUNDATION	N01CM53838	179,965
MOUNT SINAI SCHOOL OF MEDICINE	N01CM53837	60,900
NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	2,506
OHIO STATE UNIVERSITY	N01CM53828	48,000
PUBLIC HEALTH SERVICE	Y01CM40003	101,946
SIDNEY FARBER CANCER CENTER	N01CM53839	98,920
TEXAS, UNIV. OF-M.D. ANDERSON	N01CM53773	194,425
VETERANS ADMINISTRATION	Y01CM40004	353,293
WALTER REED ARMY MEDICAL CENTER	Y01CM40005	2,400
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM33711	7,350
TOTAL		1,516,925

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
PHASE II CLINICAL TRIALS:		
ARIZONA, UNIVEPSITY OF	N01CM33713	72,974
BOWMAN GRAY SCHOOL OF MEDICINE	N01CM67054	11,570
CALIFORNIA, UNIVERSITY OF	N01CM53752	36,442
CALIFORNIA, UNIVERSITY OF	N01CM67055	26,086
CAPE TOWN, UNIVERSITY OF	N01CM33738	3,260
CLINICA NEUROCHIRURGICA DELL UNIVERSI	N01CM67056	6,300
DUKE UNIVERSITY	N01CM67010	32,083
ENERGY RESEARCH & DEVELOPMENT ADMINIST	Y01CM10060	91,902
HEALTH RESEAPCH, INC.	N01CM53834	158,359
HEALTH RESEAPCH, INC.	N01CM33734	19,870
HEBREW UNIVERSITY	N01CM12127	60,000
INDIANA UNIVERSITY FOUNDATION	N01CM67057	15,575
IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM43726	21,000
IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM33714	17,968
KENTUCKY, UNIVERSITY OF	N01CM67058	20,976
LEEDS, UNIVERSITY OF	N01CM43727	37,446
MARYLAND, UNIVERSITY OF	N01CM43748	1,152,000

CONTRACT	#	DOLLAR LEVEL
PHASE II CLINICAL TRIALS:		
MAYO FOUNDATION	N01CM57044	213,989
MAYO FOUNDATION	N01CM53772	102,570
MAYO FOUNDATION	N01CM02066	103,583
MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57043	204,570
MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	38,995
MISSOURI, UNIVERSITY OF	N01CM12323	173,000
MONTEFIORE HOSPITAL	N01CM67061	16,883
MOUNT SINAI SCHOOL OF MEDICINE	N01CM53841	31,665
MOUNT SINAI SCHOOL OF MEDICINE	N01CM43703	45,000
NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	5,370
NEW YORK UNIVERSITY MEDICAL CENTER	N01CM71174	1,150
NEW YORK UNIVERSITY MEDICAL CENTER	N01CM67059	27,483
OHIO STATE UNIVERSITY	N01CM53829	6,000
OHIO STATE UNIVERSITY RESEARCH FOUNDATI	N01CM67060	14,031
OHIO STATE UNIVERSITY RESEARCH FOUNDATI	N01CM33735	10,500
ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	33,849
TENNESSEE, UNIVERSITY OF	N01CM57021	29,915
TEXAS, UNIVERSITY OF-SYSTEM CANCER CTR.	N01CM57042	217,096

CONTRACT	#	DOLLAR LEVEL
PHASE II CLINICAL TRIALS:		
TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM33737	41,370
TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM33710	20,000
VETERANS ADMINISTRATION	Y01CM40004	706,581
WALTER REED ARMY MEDICAL CENTER	Y01CM40005	11,400
YALE UNIVERSITY	N01CM53843	21,462
TOTAL		3,860,273

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
PHASE III CLINICAL TRIALS:		
ATOMIC ENERGY COMMISSION	Y01CM40101	81,866
BOWMAN GRAY SCHOOL OF MEDICINE	N01CM67054	23,138
CALIFORNIA, UNIVERSITY OF	N01CM02092	400,000
CALIFORNIA, UNIVERSITY OF	N01CM53752	72,882
CALIFORNIA, UNIVERSITY OF	N01CM67055	52,174
CAPE TOWN, UNIVERSITY OF	N01CM33738	19,560
CHILDREN'S HOSPITAL OF LOS ANGELES	N01CM53831	375,000
CLINICA NEUROCHIRURGICA DELL UNIVERSI	N01CM67056	12,600
COMMUNITY BLOOD & PLASMA SERVICE	N01CL42150	479,810
DUKE UNIVERSITY	N01CM67010	64,164
HEALTH RESEARCH, INC.	N01CM53834	67,868
HEALTH RESEARCH, INC.	N01CM33734	119,220
INDIANA UNIVERSITY FOUNDATION	N01CM67057	31,152
IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM33714	41,927
JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	14,192
KENTUCKY, UNIVERSITY OF	N01CM67058	41,952
MAKERERE UNIVERSITY COLLEGE COUNCIL	N01CM71343	62,500

CONTRACT	#	DOLLAR LEVEL
PHASE III CLINICAL TRIALS:		
MARYLAND, UNIVERSITY OF	N01CM43748	1,404,000
MAYO FOUNDATION	N01CM57044	213,990
MAYO FOUNDATION	N01CM53772	25,642
MAYO FOUNDATION	N01CM02066	34,527
MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57043	204,570
MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM57019	77,988
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM92177	2,596
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM63810	100,000
MONTEFIORE HOSPITAL	N01CM67061	33,764
NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	6,802
NEW YORK UNIVERSITY MEDICAL CENTER	N01CM71174	2,300
NEW YORK UNIVERSITY MEDICAL CENTER	N01CM67059	54,966
OHIO STATE UNIVERSITY RESEARCH FOUNDATI	N01CM67060	28,064
OHIO STATE UNIVERSITY RESEARCH FOUNDATI	N01CM33736	63,000
ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	67,700
TENNESSEE, UNIVERSITY OF	N01CM57021	59,830
TEXAS, UNIVERSITY OF-SYSTEM CANCER CTR.	N01CM57042	217,093
TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM33737	248,220

CONTRACT	#	DOLLAR LEVEL
PHASE III CLINICAL TRIALS:		
TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM33710	140,000
TEXAS, UNIVERSITY OF	N01CM53832	605,000
VERMONT, UNIVERSITY OF	N01CM33716	27,075
VETERANS ADMINISTRATION	Y01CM40004	1,766,453
WALTER REED ARMY MEDICAL CENTER	Y01CM40005	9,900
TOTAL		7,353,485

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		
ALBANY MEDICAL COLLEGE	N01CM57032	118,018
CAPE TOWN, UNIVERSITY OF	N01CM33738	9,780
HEALTH RESEARCH INC.	N01CM57034	127,763
HEALTH RESEARCH, INC.	N01CM43714	60,000
HEALTH RESEARCH, INC.	N01CM33734	59,610
IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM43726	31,500
IST. NAZION PER LO STUD E LA CURA DEI TUMORI	N01CM33714	59,895
MAKERERE UNIVERSITY COLLEGE COUNCIL	N01CM71343	62,500
MARYLAND, UNIVERSITY OF	N01CM43748	360,000
MAYO FOUNDATION	N01CM43715	38,000
MAYO FOUNDATION	N01CM57033	186,343
MEMORIAL HOSP FOR CANCER & ALLIED DISEASES	N01CM53844	105,593
MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM43716	60,500
MOUNT SINAI SCHOOL OF MEDICINE	N01CM53841	126,656
NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	2,864
OHIO STATE UNIVERSITY RESEARCH FOUNDATI	N01CM33736	31,500
SIDNEY FARBER CANCER CENTER	N01CM57035	135,132

CONTRACT	#	DOLLAR LEVEL
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		
TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM33737	124,110
TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM33710	40,000
VETERANS ADMINISTRATION	Y01CM40004	706,582
WALTER REED ARMY MEDICAL CENTER	Y01CM40005	3,900
YALE UNIVERSITY	N01CM53843	89,848
TOTAL		2,548,394

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
D.O.S. PHARMACOLOGY/TOXICOLOGY:		
ARMED FORCES RADIOBIOLOGY RESEARCH	Y01CM60104	33,709
KANSAS, UNIVERSITY OF	N01CM12184	100,000
NATIONAL NAVAL MEDICAL CENTER	Y01CM50100	358
PUBLIC HEALTH SERVICE	Y01CM40003	87,383
TOTAL		221,450

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
D.O.S. DATA PROCESSING:		
ARIZONA, UNIVERSITY OF	N01CM33713	24,325
AUTOMATION INDUSTRIES, INC.	N01CM33717	22,359
CONTROL DATA CORPORATION	N01CM33739	45,000
MASON RESEARCH INSTITUTE	N01CM67011	189,956
RES. FDN. OF STATE UNIVERSITY OF NEW YORK	N01CM33715	698,412
WALTER REED ARMY MEDICAL CENTER	Y01CM40005	2,400
TOTAL		982,452

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY

FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACT	#	DOLLAR LEVEL
D.O.S. OTHER:		
GEORGETOWN UNIVERSITY	N01CM12123	292,950
HAZLETON LABORATORIES, INC.	N01CM33705	381,545
TOTAL		674,495

TABLE III

ANALYSIS OF DIVISION OF CANCER TREATMENT

ACTIVITIES BY CONTRACT

FOR FISCAL YEAR 1976
AS OF 02/29/76

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT	DOLLAR LEVEL
ABBOTT LABORATORIES	N01CM43704		145,922
1XX ACQUISITION OF MATERIALS:		* 60*	87,554
05 BIOASSAY OF NATURAL PRODUCTS		24	35,021
98 DATA PROCESSING AND SUPPORT		16	23,348
99 OTHER-HUMAN CELL LINES		20	29,184
XXX PROGRAM MANAGEMENT:		* 40*	58,368
01 ADMINISTRATION		20	29,184
98 DATA PROCESSING AND SUPPORT		20	29,184
AGRICULTURE, DEPARTMENT OF	Y01CM40001		433,108
1XX ACQUISITION OF MATERIALS:		* 90*	389,797
02 NEW AGENT PROCUREMENT, PLANT PRODUCTS		85	368,142
98 DATA PROCESSING AND SUPPORT		5	21,655
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	43,311
02 PLANT PRODUCTS			
ALBANY MEDICAL COLLEGE	N01CM57032		118,018
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		*100*	118,018
06 COLON 20 CHEM.		30	35,405
06 COLON 21 SURG.		30	35,405
06 COLON 22 RAD.		10	11,802
06 COLON 23 IMM.		30	35,405
ALDRICH CHEMICAL COMPANY, INC.	N01CM23706		191,815
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 70*	134,270
01 SYNTHETICS			
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 30*	57,545
01 PRODUCTION, SYNTHETICS			
ALLEGHENY GENERAL HOSPITAL	N01CM43730		89,718
2B2 VERIFICATION SCREEN:		* 75*	67,288
01 DETAILED DRUG EVALUATION, IN VITRO		25	22,430

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
02 DETAILED DRUG EVALUATION, IN VIVO		50	44,859	
3X2 PRECLIN. COMBINED TREATMENT:		* 25*	22,430	
02 COMBINED MODALITY PROGRAM				
ARIZONA, UNIVERSITY OF	N01CM33713			121,624
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 20*	24,325	
04 MARKERS				
PHASE II CLINICAL TRIALS:		* 60*	72,974	
16 MYELOMA	12 MARKERS			
D.O.S. DATA PROCESSING:		* 20*	24,325	
16 MYELOMA	25 DATA PROCESSING			
ARMED FORCES RADIOBIOLOGY	Y01CM60104			33,709
D.O.S. PHARMACOLOGY/TOXICOLOGY:		*100*	33,709	
01 NONSPECIFIC	24 PHARM./TOX.			
ARMY, DEPARTMENT OF, FT. DETRICK	Y01CO50004			416,672
1XX ACQUISITION OF MATERIALS:		* 48*	200,003	
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS				
2A1 BASIC SCREEN:		* 12*	50,000	
01 PRIMARY SCREEN:ING, IN VITRO		3	12,500	
04 ANIMALS		9	37,500	
2B2 VERIFICATION SCREEN:		* 30*	125,002	
04 ANIMALS				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	41,667	
03 FERMENTATION/ANTIBIOTICS				
ARS/SPRAGUE-DAWLEY	N01CM53813			813,951
1XX ACQUISITION OF MATERIALS:		* 20*	162,790	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	447,673	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	203,488	
04 ANIMALS				
ARS/SPRAGUE-DAWLEY	N01CM53846			215,143
1XX ACQUISITION OF MATERIALS:		* 20*	43,028	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	118,329	
04 ANIMALS				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
2B2 VERIFICATION SCREEN:		* 25*	53,786	
04 ANIMALS				
ARTHUR D. LITTLE, INC.	N01CM53849			1,228,108
2A1 BASIC SCREEN:		* 8*	98,249	
99 OTHER-TUMOR BANK				
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 15*	184,216	
03 BIOCHEMISTRY				
3X1 PHARMACOLOGY/TOXICOLOGY:		* 77*	945,643	
01 PHARMACOLOGY				
ARTHUR D. LITTLE, INC.	N01CM67078			210,267
1XX ACQUISITION OF MATERIALS:		* 5*	10,514	
05 BIOASSAY OF NATURAL PRODUCTS				
2A1 BASIC SCREEN:		* 95*	199,753	
01 PRIMARY SCREENING, IN VITRO		90	189,240	
03 PRIMARY SCREENING, REL. NEW MODEL DEVEL.		5	10,513	
ARTHUR D. LITTLE, INC.	N01CM63823			293,500
1XX ACQUISITION OF MATERIALS:		* 11*	32,285	
99 OTHER-STRUCTURE ACTIVITY				
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 62*	181,970	
01 MOLECULAR BIOLOGY		20	58,700	
02 CELL KINETICS		15	44,025	
05 BLOOD PRODUCTS		12	35,220	
99 OTHER-		15	44,025	
XXX PROGRAM MANAGEMENT:		* 15*	44,025	
01 ADMINISTRATION				
PHASE I CLINICAL TRIALS:		* 12*	35,220	
18 PANCREAS	05 PHARM./TOX.			
ARTHUR D. LITTLE, INC.	N01CM67047			118,916
2B2 VERIFICATION SCREEN:		*100*	118,916	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.				
ARTHUR D. LITTLE, INC.	N01CM53765			770,967
2A1 BASIC SCREEN:		* 35*	269,839	
02 PRIMARY SCREENING, IN VIVO				
2B2 VERIFICATION SCREEN:		* 20*	154,193	
02 DETAILED DRUG EVALUATION, IN VIVO				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
3X2 PRECLIN. COMBINED TREATMENT:		* 45*	346,935	
01 COMBINATION CHEMOTHERAPY		27	208,161	
02 COMBINED MODALITY PROGRAM		18	138,774	
ARTHUR D. LITTLE, INC.	N01CM33727			165,344
1XX ACQUISITION OF MATERIALS:		* 16*	26,457	
05 BIOASSAY OF NATURAL PRODUCTS				
2A1 BASIC SCREEN:		* 40*	66,137	
01 PRIMARY SCREEN:ING, IN VITRO		5	8,267	
02 PRIMARY SCREEN:ING, IN VIVO		13	21,495	
03 PRIMARY SCREEN:ING, REL. NEW MODEL DEVEL.		2	3,307	
98 DATA PROCESSING AND SUPPORT		19	31,415	
99 OTHER- TUMOR BANK		1	1,653	
2B2 VERIFICATION SCREEN:		* 40*	66,137	
01 DETAILED DRUG EVALUATION, IN VITRO		3	4,960	
02 DETAILED DRUG EVALUATION, IN VIVO		26	42,989	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		11	18,188	
3X2 PRECLIN. COMBINED TREATMENT:		* 4*	6,613	
01 COMBINATION CHEMOTHERAPY		3	4,960	
02 COMBINED MODALITY PROGRAM		1	1,653	
ASH STEVENS, INC.	N01CM12314			2,816
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 75*	2,112	
01 SYNTHETICS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 25*	704	
01 PRODUCTION, SYNTHETICS				
ASH STEVENS, INC.	N01CM57012			214,209
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 75*	160,657	
01 SYNTHETICS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 25*	53,552	
01 PRODUCTION, SYNTHETICS				
ASSOCIATED BIOMEDIC SYSTEMS, INC.	N01CM02271			272,178
1XX ACQUISITION OF MATERIALS:		* 80*	217,742	
99 OTHER-HUMAN BLOOD				
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 10*	27,218	
01 MOLECULAR BIOLOGY				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	27,218	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
06 ANALYTICAL AND QUALITY CONTROL				
ATOMIC ENERGY COMMISSION	Y01CM40101			81,866
PHASE III CLINICAL TRIALS:		*100*	81,866	
01 NONSPECIFIC	17 BLOOD PROD.			
ATOMIC ENERGY COMMISSION	Y01CM10056			25,500
2B2 VERIFICATION SCREEN:		*100*	25,500	
01 DETAILED DRUG EVALUATION, IN VITRO		70	17,850	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		30	7,650	
AUTOMATION INDUSTRIES, INC.	N01CM33717			22,359
D.O.S. DATA PROCESSING:		*100*	22,359	
04 BREAST	25 DATA PROCESSING	35	7,826	
12 LEUKEMIA	25 DATA PROCESSING	35	7,826	
14 LYMPHOMAS	25 DATA PROCESSING	8	1,789	
15 MELANOMA	25 DATA PROCESSING	9	2,012	
17 OVARY	25 DATA PROCESSING	8	1,789	
19 PEDIATRIC-LYMPHOMA,SARCOMA	25 DATA PROCESSING	5	1,118	
BATTELLE MEMORIAL INSTITUTE	N01CM43746			1,500,000
3X1 PHARMACOLOGY/TOXICOLOGY:		*100*	1,500,000	
02 PROTOCOL TOXICITY STUDIES		50	750,000	
03 SPECIAL TOXICITY STUDIES		34	510,000	
04 ANIMALS		1	15,000	
98 DATA PROCESSING AND SUPPORT		15	225,000	
BAYLOR COLLEGE OF MEDICINE	N01CM57018			34,000
2B2 VERIFICATION SCREEN:		*100*	34,000	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.				
BELLAIRE ACRES	N01CM50583			209,040
1XX ACQUISITION OF MATERIALS:		* 20*	41,808	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	114,972	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	52,260	
04 ANIMALS				
BEN VENUE LABORATORIES, INC.	N01CM53811			631,028
2B1 FORMULATION:		* 10*	63,103	
01 DEVEL. OF EXPERIMENTAL FORMULATIONS				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 90*	567,925	
06 FORMULATION		75	473,271	
07 ANALYTICAL AND QUALITY CONTROL		15	94,654	
BOWMAN GRAY SCHOOL OF MEDICINE	N01CM67054			38,564
XXX PROGRAM MANAGEMENT:		* 10*	3,856	
01 ADMINISTRATION				
PHASE II CLINICAL TRIALS:		* 30*	11,570	
03 BRAIN	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 60*	23,138	
03 BRAIN	13 CHEM.	30	11,569	
03 BRAIN	15 RAD.	30	11,569	
BRIGHAM YOUNG UNIVERSITY	N01CM43790			81,019
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 30*	24,305	
03 BIOCHEMISTRY				
2B2 VERIFICATION SCREEN:		* 70*	56,714	
01 DETAILED DRUG EVALUATION, IN VITRO		40	32,408	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		30	24,306	
BRISTOL-MYERS COMPANY	N01CM43759			223,578
1XX ACQUISITION OF MATERIALS:		*100*	223,578	
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS				
BRITISH COLUMBIA, UNIVERSITY OF	N01CM23223			92,379
1XX ACQUISITION OF MATERIALS:		* 80*	73,903	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 5*	4,619	
05 RADIOLABELED MATERIALS				
3X1 PHARMACOLOGY/TOXICOLOGY:		* 15*	13,857	
99 OTHER-METABOLITE ISOLATION				
CALIFORNIA, UNIVERSITY OF	N01CM02092			400,000
PHASE III CLINICAL TRIALS:		*100*	400,000	
12 LEUKEMIA	18 TRANSF. RESEARCH	80	320,000	
14 LYMPHOMAS	18 TRANSF. RESEARCH	10	40,000	
99 OTHER-APLASTIC	18 TRANSF. RESEARCH	10	40,000	
CALIFORNIA, UNIVERSITY OF	N01CM43791			92,811
2B2 VERIFICATION SCREEN:		* 50*	46,405	
01 DETAILED DRUG EVALUATION, IN VITRO		20	18,562	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT	DOLLAR LEVEL
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		30	27,843
3X2 PRECLIN. COMBINED TREATMENT:		* 50*	46,406
02 COMBINED MODALITY PROGRAM			
CALIFORNIA, UNIVERSITY OF	N01CM53752		121,471
XXX PROGRAM MANAGEMENT:		* 10*	12,147
01 ADMINISTRATION			
PHASE II CLINICAL TRIALS:		* 30*	36,442
03 BRAIN	07 CHEM.		
PHASE III CLINICAL TRIALS:		* 60*	72,882
03 BRAIN	13 CHEM.	30	36,441
03 BRAIN	15 RAD.	30	36,441
CALIFORNIA, UNIVERSITY OF	N01CM67055		86,956
XXX PROGRAM MANAGEMENT:		* 10*	8,696
01 ADMINISTRATION			
PHASE II CLINICAL TRIALS:		* 30*	26,086
03 BRAIN	07 CHEM.		
PHASE III CLINICAL TRIALS:		* 60*	52,174
03 BRAIN	13 CHEM.	30	26,087
03 BRAIN	15 RAD.	30	26,087
CAPE TOWN, UNIVERSITY OF	N01CM33738		32,600
PHASE II CLINICAL TRIALS:		* 10*	3,260
13 LUNG	07 CHEM.		
PHASE III CLINICAL TRIALS:		* 60*	19,560
13 LUNG	13 CHEM.	20	6,520
13 LUNG	14 SURG.	20	6,520
13 LUNG	15 RAD.	20	6,520
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 30*	9,780
13 LUNG	20 CHEM.	15	4,890
13 LUNG	21 SURG.	15	4,890
CATHOLIC MEDICAL CTR OF BROOKLYN AND	N01CM23703		450,000
2A1 BASIC SCREEN:		* 10*	45,000
02 PRIMARY SCREENING, IN VIVO			
2B2 VERIFICATION SCREEN:		* 55*	247,500
02 DETAILED DRUG EVALUATION, IN VIVO		50	225,000
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		5	22,500

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
3X2 PRECLIN. COMBINED TREATMENT:		* 35*	157,500	
01 COMBINATION CHEMOTHERAPY		25	112,500	
02 COMBINED MODALITY PROGRAM		10	45,000	
CHARLES RIVER BREEDING LABS.	N01CM53848			205,234
1XX ACQUISITION OF MATERIALS:		* 20*	41,046	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	112,879	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	51,309	
04 ANIMALS				
CHARLES RIVER BREEDING LABS.	N01CM50598			226,200
1XX ACQUISITION OF MATERIALS:		* 20*	45,240	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	124,410	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	56,550	
04 ANIMALS				
CHARLES RIVER BREEDING LABS.	N01CM53812			1,169,935
1XX ACQUISITION OF MATERIALS:		* 20*	233,987	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	643,464	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	292,484	
04 ANIMALS				
CHEMICAL ABSTRACTS SERVICE	N01CM43722			452,000
1XX ACQUISITION OF MATERIALS:		*100*	452,000	
98 DATA PROCESSING AND SUPPORT				
CHESTER BEATTY RESEARCH INSTITUTE	N01CM43736			32,000
1XX ACQUISITION OF MATERIALS:		* 50*	16,000	
01 NEW AGENT PROCUREMENT, SYNTHETICS		40	12,800	
98 DATA PROCESSING AND SUPPORT		10	3,200	
2B2 VERIFICATION SCREEN:		* 50*	16,000	
01 DETAILED DRUG EVALUATION, IN VITRO		5	1,600	
02 DETAILED DRUG EVALUATION, IN VIVO		20	6,400	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		25	8,000	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
CHILDREN'S HOSPITAL OF LOS ANGELES	N01CM53831			375,000
PHASE III CLINICAL TRIALS:		*100*	375,000	
19 PEDIATRIC-NEUROBL. & RHABDO	19 PROTECT. ENV.			
CLINICA NEUROCHIRURGICA DEL	N01CM67056			21,000
XXX PROGRAM MANAGEMENT:		* 10*	2,100	
01 ADMINISTRATION				
PHASE II CLINICAL TRIALS:		* 30*	6,300	
03 BRAIN	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 60*	12,600	
03 BRAIN	13 CHEM.	30	6,300	
03 BRAIN	15 RAD.	30	6,300	
COLLABORATIVE RESEARCH, INC.	N01CM60506			45,000
1XX ACQUISITION OF MATERIALS:		* 90*	40,500	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	4,500	
01 SYNTHETICS				
COLLABORATIVE RESEARCH, INC.	N01CM12326			80,000
1XX ACQUISITION OF MATERIALS:		*100*	80,000	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
COMMUNITY BLOOD & PLASMA SERVICE	N01CL42150			479,810
PHASE III CLINICAL TRIALS:		*100*	479,810	
12 LEUKEMIA	17 BLOOD PROD.	80	383,848	
14 LYMPHOMAS	17 BLOOD PROD.	10	47,981	
99 OTHER-APLASIA	17 BLOOD PROD.	10	47,981	
CONTROL DATA CORPORATION	N01CM33739			50,000
XXX PROGRAM MANAGEMENT:		* 10*	5,000	
01 ADMINISTRATION				
D.O.S. DATA PROCESSING:		* 90*	45,000	
03 BRAIN	25 DATA PROCESSING			
CORDOVA CHEMICAL COMPANY	N01CM57031			243,413
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 25*	60,853	
01 SYNTHETICS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 75*	182,560	
01 PRODUCTION, SYNTHETICS				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
DOT SYSTEMS, INC.	N01CM53774			150,000
XXX PROGRAM MANAGEMENT:		*100*	150,000	
02 COMMUNICATION AND EDUCATION				
DOW CHEMICAL COMPANY	N01CM53768			243,008
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 25*	60,752	
01 SYNTHETICS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 75*	182,256	
01 PRODUCTION, SYNTHETICS				
DUKE UNIVERSITY	N01CM67010			106,941
XXX PROGRAM MANAGEMENT:		* 10*	10,694	
01 ADMINISTRATION				
PHASE II CLINICAL TRIALS:		* 30*	32,083	
03 BRAIN	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 60*	64,164	
03 BRAIN	13 CHEM.	30	32,082	
03 BRAIN	15 RAD.	30	32,082	
ENERGY RESEARCH & DEVELOPMENT	Y01CM10060			91,902
PHASE II CLINICAL TRIALS:		*100*	91,902	
01 NONSPECIFIC	12 MARKERS			
FARMITALIA	N01CM57036			175,622
1XX ACQUISITION OF MATERIALS:		* 90*	158,060	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	17,562	
01 SYNTHETICS				
FARMITALIA	N01CM57014			179,082
1XX ACQUISITION OF MATERIALS:		* 90*	161,174	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	17,908	
01 SYNTHETICS				
FLOW LABORATORIES	N01CM50571			218,400
1XX ACQUISITION OF MATERIALS:		* 20*	43,680	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	120,120	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	54,600	
04 ANIMALS				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
GEORGE WASHINGTON UNIVERSITY	N01CM12206			71,641
1XX ACQUISITION OF MATERIALS:		* 20*	14,328	
06 ANIMALS				
3X1 PHARMACOLOGY/TOXICOLOGY:		* 80*	57,313	
01 PHARMACOLOGY				
GEORGETOWN UNIVERSITY	N01CM12123			315,000
XXX PROGRAM MANAGEMENT:		* 7*	22,050	
01 ADMINISTRATION		2	6,300	
98 DATA PROCESSING AND SUPPORT		5	15,750	
D.O.S. OTHER:		* 93*	292,950	
01 NONSPECIFIC	99 OTHER-MICROBIOLOGY	10	31,500	
03 BRAIN	99 OTHER-MICROBIOLOGY	5	15,750	
06 COLON	99 OTHER-MICROBIOLOGY	6	18,900	
11 KIDNEY	99 OTHER-MICROBIOLOGY	5	15,750	
12 LEUKEMIA	99 OTHER-MICROBIOLOGY	33	103,950	
13 LUNG	99 OTHER-MICROBIOLOGY	5	15,750	
14 LYMPHOMAS	99 OTHER-MICROBIOLOGY	19	59,850	
21 SARCOMAS	99 OTHER-MICROBIOLOGY	5	15,750	
23 TESTICULAR	99 OTHER-MICROBIOLOGY	5	15,750	
GEORGETOWN UNIVERSITY	N01CM33745			895,883
XXX PROGRAM MANAGEMENT:		*100*	895,883	
01 ADMINISTRATION				
GEORGETOWN UNIVERSITY	N01CM43732			64,882
1XX ACQUISITION OF MATERIALS:		*100*	64,882	
01 NEW AGENT PROCUREMENT, SYNTHETICS		25	16,221	
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS		75	48,662	
HARLAN INDUSTRIES	N01CM23218	-		43,105
1XX ACQUISITION OF MATERIALS:		* 20*	8,621	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	23,708	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	10,776	
04 ANIMALS				
HARLAN INDUSTRIES	N01CM50591			219,960
1XX ACQUISITION OF MATERIALS:		* 20*	43,992	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	120,978	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	54,990	
04 ANIMALS				
HARVARD UNIVERSITY	N01CM53825			58,154
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 70*	40,708	
01 MOLECULAR BIOLOGY				
2B2 VERIFICATION SCREEN:		* 30*	17,446	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.				
HAWAII, UNIVERSITY OF	N01CM33747			84,000
1XX ACQUISITION OF MATERIALS:		*100*	84,000	
02 NEW AGENT PROCUREMENT, PLANT PRODUCTS				
HAZLETON LABORATORIES, INC.	N01CM43702			408,580
2A2 BIOLOGICAL RESEARCH PROGRAMS:		*100*	408,580	
06 TRANS. RESEARCH		50	204,290	
99 OTHER-TRANSPLANTATION		50	204,290	
HAZLETON LABORATORIES, INC.	N01CM43705			33,000
3X1 PHARMACOLOGY/TOXICOLOGY:		* 97*	32,010	
03 SPECIAL TOXICITY STUDIES				
XXX PROGRAM MANAGEMENT:		* 3*	990	
01 ADMINISTRATION				
HAZLETON LABORATORIES, INC.	N01CM33705			410,263
XXX PROGRAM MANAGEMENT:		* 7*	28,718	
01 ADMINISTRATION		2	8,205	
98 DATA PROCESSING AND SUPPORT		5	20,513	
D.O.S. OTHER:		* 93*	381,545	
01 NONSPECIFIC	99 OTHER-VIROLOGY	10	41,026	
03 BRAIN	99 OTHER-VIROLOGY	5	20,513	
06 COLON	99 OTHER-VIROLOGY	6	24,616	
11 KIDNEY	99 OTHER-VIROLOGY	5	20,513	
12 LEUKEMIA	99 OTHER-VIROLOGY	33	135,387	
13 LUNG	99 OTHER-VIROLOGY	5	20,513	
14 LYMPHOMAS	99 OTHER-VIROLOGY	19	77,950	
21 SARCOMAS	99 OTHER-VIROLOGY	5	20,513	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
23 TESTICULAR	99 OTHER-VIROLOGY	5	20,513	
HAZLETON LABORATORIES, INC.	N01CM33708			381,970
1XX ACQUISITION OF MATERIALS:		* 20*	76,394	
06 ANIMALS				
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 30*	114,591	
04 MARKERS				
2B2 VERIFICATION SCREEN:		* 10*	38,197	
04 ANIMALS				
3X1 PHARMACOLOGY/TOXICOLOGY:		* 40*	152,788	
01 PHARMACOLOGY		10	38,197	
03 SPECIAL TOXICITY STUDIES		30	114,591	
HAZLETON LABORATORIES, INC.	N01CM23701			147,614
2A2 BIOLOGICAL RESEARCH PROGRAMS:		*100*	147,614	
99 OTHER-IMMUNOLOGY				
HAZLETON LABORATORIES, INC.	N01CM57007			8,158
1XX ACQUISITION OF MATERIALS:		* 15*	1,223	
05 BIOASSAY OF NATURAL PRODUCTS				
2A1 BASIC SCREEN:		* 60*	4,895	
02 PRIMARY SCREEN:ING, IN VIVO		55	4,487	
03 PRIMARY SCREEN:ING, REL. NEW MODEL DEVEL.		5	408	
2B2 VERIFICATION SCREEN:		* 22*	1,795	
02 DETAILED DRUG EVALUATION, IN VIVO				
3X2 PRECLIN. COMBINED TREATMENT:		* 3*	245	
01 COMBINATION CHEMOTHERAPY				
HAZLETON RESEARCH ANIMALS, INC.	N01CM60125			40,500
3X1 PHARMACOLOGY/TOXICOLOGY:		*100*	40,500	
04 ANIMALS				
HAZLETON RESEARCH ANIMALS, INC.	N01CM41150			1,324
3X1 PHARMACOLOGY/TOXICOLOGY:		*100*	1,324	
04 ANIMALS				
HEALTH RESEARCH INC.	N01CM57034			127,763
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		*100*	127,763	
06 COLON	20 CHEM.	30	38,329	
06 COLON	21 SURG.	30	38,329	
06 COLON	22 RAD.	10	12,776	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
06 COLON	23 IMM.	30	38,329	
HEALTH RESEARCH, INC.	N01CM57039			95,016
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 40*	38,006	
99 OTHER-IMMUNOLOGY				
2B2 VERIFICATION SCREEN:		* 60*	57,010	
01 DETAILED DRUG EVALUATION, IN VITRO		30	28,505	
02 DETAILED DRUG EVALUATION, IN VIVO		30	28,505	
HEALTH RESEARCH, INC.	N01CM53834			226,227
PHASE II CLINICAL TRIALS:		* 70*	158,359	
04 BREAST	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 30*	67,868	
04 BREAST	13 CHEM.			
HEALTH RESEARCH, INC.	N01CM43714			60,000
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		*100*	60,000	
23 TESTICULAR	20 CHEM.	33	19,800	
23 TESTICULAR	21 SURG.	33	19,800	
23 TESTICULAR	22 RAD.	34	20,400	
HEALTH RESEARCH, INC.	N01CM33734			198,700
PHASE II CLINICAL TRIALS:		* 10*	19,870	
13 LUNG	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 60*	119,220	
13 LUNG	13 CHEM.	20	39,740	
13 LUNG	14 SURG.	20	39,740	
13 LUNG	15 RAD.	20	39,740	
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 30*	59,610	
13 LUNG	20 CHEM.	15	29,805	
13 LUNG	21 SURG.	15	29,805	
HEALTH RESEARCH, INC.	N01CM33726			17,924
2A1 BASIC SCREEN:		* 80*	14,339	
01 PRIMARY SCREEN:ING, IN VITRO		60	10,754	
02 PRIMARY SCREEN:ING, IN VIVO		20	3,585	
3X1 PHARMACOLOGY/TOXICOLOGY:		* 20*	3,585	
01 PHARMACOLOGY				
HEALTH RESEARCH, INC.	N01CM02035			151,564
1XX ACQUISITION OF MATERIALS:		* 20*	30,313	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	83,360	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	37,891	
04 ANIMALS				
HEALTH RESEARCH, INC.	N01CM43785			57,210
2B2 VERIFICATION SCREEN:		*100*	57,210	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.				
HEBREW UNIVERSITY	N01CM12127			120,000
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 50*	60,000	
99 OTHER-IMMUNOLOGY				
PHASE II CLINICAL TRIALS:		* 50*	60,000	
12 LEUKEMIA	10 IMM.	25	30,000	
12 LEUKEMIA	11 PHARM./TOX.	25	30,000	
IIT RESEARCH INSTITUTE	N01CM53829			122,007
1XX ACQUISITION OF MATERIALS:		* 20*	24,401	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	67,104	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	30,502	
04 ANIMALS				
IIT RESEARCH INSTITUTE	N01CM43755			224,599
1XX ACQUISITION OF MATERIALS:		* 20*	44,920	
05 BIOASSAY OF NATURAL PRODUCTS				
2A1 BASIC SCREEN:		* 65*	145,989	
01 PRIMARY SCREEN:ING, IN VITRO		5	11,230	
02 PRIMARY SCREEN:ING, IN VIVO		55	123,529	
98 DATA PROCESSING AND SUPPORT		* 5	11,230	
2B2 VERIFICATION SCREEN:		* 15*	33,690	
02 DETAILED DRUG EVALUATION, IN VIVO				
IIT RESEARCH INSTITUTE	N01CM63832			77,405
1XX ACQUISITION OF MATERIALS		* 5*	3,870	
05 BIOASSAY OF NATURAL PRODUCTS				
2A1 BASIC SCREEN:		* 95*	73,535	
01 PRIMARY SCREEN:ING, IN VITRO		90	69,665	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
KANSAS, UNIVERSITY OF	N01CM53819			103,531
1XX ACQUISITION OF MATERIALS:		* 20*	20,706	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	56,942	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	25,883	
04 ANIMALS				
KANSAS, UNIVERSITY OF	N01CM23217			100,000
2B1 FORMULATION:		*100*	100,000	
01 DEVEL. OF EXPERIMENTAL FORMULATIONS				
KENTUCKY, UNIVERSITY OF	N01CM67058			69,920
XXX PROGRAM MANAGEMENT:		* 10*	6,992	
01 ADMINISTRATION				
PHASE II CLINICAL TRIALS:		* 30*	20,976	
03 BRAIN	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 60*	41,952	
03 BRAIN	13 CHEM.	30	20,976	
03 BRAIN	15 RAD.	30	20,976	
LABORATORY RESEARCH ENTERPRISES, INC.	N01CM60124			39,750
3X1 PHARMACOLOGY/TOXICOLOGY:		*100*	39,750	
04 ANIMALS				
LABORATORY SUPPLY CO.	N01CM50577			219,960
1XX ACQUISITION OF MATERIALS:		* 20*	43,992	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	120,978	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	54,990	
04 ANIMALS				
LABORATORY SUPPLY CO.	N01CM53775			129,731
1XX ACQUISITION OF MATERIALS:		* 20*	25,946	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	71,352	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	32,433	
04 ANIMALS				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
LEEDS, UNIVERSITY OF	N01CM43727			37,446
PHASE II CLINICAL TRIALS:		*100*	37,446	
06 COLON	07 CHEM.	50	18,723	
22 STOMACH	07 CHEM.	50	18,723	
LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM53814			442,404
1XX ACQUISITION OF MATERIALS:		* 20*	88,481	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	243,322	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	110,601	
04 ANIMALS				
LITTON BIONETICS, INC.	N01CM33741			77,000
2A1 BASIC SCREEN:		* 30*	23,100	
01 PRIMARY SCREEN:ING, IN VITRO		10	7,700	
03 PRIMARY SCREEN:ING, REL. NEW MODEL DEVEL.		20	15,400	
2B2 VERIFICATION SCREEN:		* 70*	53,900	
01 DETAILED DRUG EVALUATION, IN VITRO		50	38,500	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		20	15,400	
LITTON BIONETICS, INC.	N01CM22062			278,853
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 30*	83,656	
01 MOLECULAR BIOLOGY				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 70*	195,197	
99 OTHER-EMBRYONIC CELLS				
LITTON BIONETICS, INC.	N01CM12340			1,410,419
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 50*	705,209	
01 MOLECULAR BIOLOGY				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 50*	705,210	
99 OTHER-ANTIBODIES/ANTIGENS				
LITTON BIONETICS, INC.	N01CP33211			50,000
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 50*	25,000	
01 MOLECULAR BIOLOGY				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 50*	25,000	
99 OTHER-ANTIBODIES/ANTIGENS				
LITTON BIONETICS, INC.	N01CO25423			4,148,460
1XX ACQUISITION OF MATERIALS:		* 48*	1,991,261	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS				
2A1 BASIC SCREEN:		* 12*	497,815	
01 PRIMARY SCREEN:ING, IN VITRO		3	124,454	
04 ANIMALS		9	373,361	
2B2 VERIFICATION SCREEN:		* 30*	1,244,538	
04 ANIMALS				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	414,846	
03 FERMENTATION/ANTIBIOTICS				
LITTON BIONETICS, INC.	N01CM53778			71,172
1XX ACQUISITION OF MATERIALS:		* 20*	14,234	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	39,145	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	17,793	
04 ANIMALS				
MADRID, UNIVERSITY OF	N01CM53792			30,016
2B2 VERIFICATION SCREEN:		*100*	30,016	
01 DETAILED DRUG EVALUATION, IN VITRO		70	21,011	
02 DETAILED DRUG EVALUATION, IN VIVO		10	3,002	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		20	6,003	
MAKERERE UNIVERSITY COLLEGE COUNCIL	N01CM71343			125,000
PHASE III CLINICAL TRIALS:		* 50*	62,500	
10 HEPATOMA 13 CHEM.		40	50,000	
10 HEPATOMA 14 SURG.		10	12,500	
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 50*	62,500	
14 LYMPHOMAS 20 CHEM.		40	50,000	
14 LYMPHOMAS 22 RAD.		10	12,500	
MARIO NEGRI INST OF PHARMACOLOGICAL	N01CM53826			86,436
3X2 PRECLIN. COMBINED TREATMENT:		*100*	86,436	
02 COMBINED MODALITY PROGRAM				
MARIO NEGRI INST OF PHARMACOLOGICAL	N01CM33720			179,100
1XX ACQUISITION OF MATERIALS:		* 15*	26,865	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2A1 BASIC SCREEN:		* 70*	125,370	
02 PRIMARY SCREEN:ING, IN VIVO				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT	DOLLAR LEVEL
2B2 VERIFICATION SCREEN:		* 15*	26,865
02 DETAILED DRUG EVALUATION, IN VIVO		10	17,910
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		5	8,955
MARSHALL RESEARCH ANIMALS, INC.	N01CM60123		42,000
3X1 PHARMACOLOGY/TOXICOLOGY:		*100*	42,000
04 ANIMALS			
MARYLAND, UNIVERSITY OF	N01CM43748		3,600,000
XXX PROGRAM MANAGEMENT:		* 7*	252,000
01 ADMINISTRATION			
PHASE I CLINICAL TRIALS:		* 12*	432,000
01 NONSPECIFIC	04 IMM.	5	180,000
01 NONSPECIFIC	05 PHARM./TOX.	5	180,000
03 BRAIN	01 CHEM.	2	72,000
PHASE II CLINICAL TRIALS:		* 32*	1,152,000
03 BRAIN	07 CHEM.	2	72,000
06 COLON	07 CHEM.	5	180,000
11 KIDNEY	07 CHEM.	5	180,000
12 LEUKEMIA	07 CHEM.	5	180,000
12 LEUKEMIA	10 IMM.	5	180,000
13 LUNG	10 IMM.	5	180,000
23 TESTICULAR	07 CHEM.	5	180,000
PHASE III CLINICAL TRIALS:		* 39*	1,404,000
03 BRAIN	13 CHEM.	1	36,000
12 LEUKEMIA	13 CHEM.	6	216,000
12 LEUKEMIA	18 TRANSF. RESEARCH	10	360,000
12 LEUKEMIA	19 PROTECT. ENV.	5	180,000
14 LYMPHOMAS	13 CHEM.	7	252,000
14 LYMPHOMAS	19 PROTECT. ENV.	5	180,000
21 SARCOMAS	13 CHEM.	5	180,000
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 10*	360,000
12 LEUKEMIA	23 IMM.	2	72,000
14 LYMPHOMAS	20 CHEM.	5	180,000
14 LYMPHOMAS	23 IMM.	2	72,000
15 MELANOMA	23 IMM.	1	36,000

CONTRACTOR/FUNCTIONS	#		PERCENT OF EFFORT		DOLLAR LEVEL
MASON RESEARCH INSTITUTE	N01CM57030				155,488
2B2 VERIFICATION SCREEN:			*100*	155,488	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.					
MASON RESEARCH INSTITUTE	N01CM67011				189,956
D.O.S. DATA PROCESSING:			*100*	189,956	
04 BREAST	25	DATA PROCESSING	35	66,485	
12 LEUKEMIA	25	DATA PROCESSING	35	66,485	
14 LYMPHOMAS	25	DATA PROCESSING	5	9,498	
15 MELANOMA	25	DATA PROCESSING	15	28,493	
21 SARCOMAS	25	DATA PROCESSING	5	9,498	
99 OTHER-	25	DATA PROCESSING	5	9,498	
MASSACHUSETTS INSTITUTE OF	N01CM43712				60,643
1XX ACQUISITION OF MATERIALS:			*100*	60,643	
01 NEW AGENT PROCUREMENT, SYNTHETICS					
MAYO FOUNDATION	N01CM43715				38,000
PHASE IV CLINICAL TRIALS (COMB. MODAL.):			*100*	38,000	
23 TESTICULAR	20	CHEM.	33	12,540	
23 TESTICULAR	21	SURG.	34	12,920	
23 TESTICULAR	22	RAD.	33	12,540	
MAYO FOUNDATION	N01CM57044				427,979
PHASE II CLINICAL TRIALS:			* 50*	213,989	
02 BLADDER	07	CHEM.	2	8,560	
04 BREAST	07	CHEM.	5	21,399	
05 CERVIX	07	CHEM.	2	8,560	
06 COLON	07	CHEM.	5	21,399	
09 HEAD & NECK	07	CHEM.	2	8,560	
11 KIDNEY	07	CHEM.	3	12,839	
13 LUNG	07	CHEM.	5	21,399	
15 MELANOMA	07	CHEM.	3	12,839	
17 OVARY	07	CHEM.	5	21,399	
18 PANCREAS	07	CHEM.	5	21,399	
20 PROSTATE	07	CHEM.	5	21,399	
21 SARCOMAS	07	CHEM.	5	21,399	
22 STOMACH	07	CHEM.	3	12,839	
PHASE III CLINICAL TRIALS:			* 50*	213,990	
02 BLADDER	13	CHEM.	3	12,839	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
04 BREAST	13 CHEM.	5	21,399	
05 CERVIX	13 CHEM.	3	12,839	
06 COLON	13 CHEM.	5	21,399	
09 HEAD & NECK	13 CHEM.	3	12,839	
11 KIDNEY	13 CHEM.	2	8,560	
13 LUNG	13 CHEM.	5	21,399	
15 MELANOMA	13 CHEM.	2	8,560	
17 OVARY	13 CHEM.	5	21,399	
18 PANCREAS	13 CHEM.	5	21,399	
20 PROSTATE	13 CHEM.	5	21,399	
21 SARCOMAS	13 CHEM.	5	21,399	
22 STOMACH	13 CHEM.	2	8,560	
MAYO FOUNDATION	N01CM57033			188,343
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		*100*	188,343	
06 COLON	20 CHEM.	30	56,503	
06 COLON	21 SURG.	30	56,503	
06 COLON	22 RAD.	10	18,834	
06 COLON	23 IMM.	30	56,503	
MAYO FOUNDATION	N01CM53838			179,965
PHASE I CLINICAL TRIALS:		*100*	179,965	
01 NONSPECIFIC	01 CHEM.			
MAYO FOUNDATION	N01CM53772			128,212
PHASE II CLINICAL TRIALS:		* 80*	102,570	
04 BREAST	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 20*	25,642	
04 BREAST	13 CHEM.			
MAYO FOUNDATION	N01CM02066			138,110
PHASE II CLINICAL TRIALS:		* 75*	103,583	
06 COLON	07 CHEM.	60	82,866	
18 PANCREAS	07 CHEM.	7	9,668	
22 STOMACH	07 CHEM.	8	11,049	
PHASE III CLINICAL TRIALS:		* 25*	34,527	
06 COLON	13 CHEM.	20	27,622	
18 PANCREAS	13 CHEM.	3	4,143	
22 STOMACH	13 CHEM.	2	2,762	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT	DOLLAR LEVEL
EMORIAL HOSP FOR CANCER & ALLIED	N01CM53844		105,593
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		*100*	105,593
06 COLON	20 CHEM.	30	31,678
06 COLON	21 SURG.	30	31,678
06 COLON	22 RAD.	10	10,559
06 COLON	23 IMM.	30	31,678
EMORIAL HOSP. FOR CANCER & ALLIED	N01CM57043		409,140
		* 50*	204,570
PHASE II CLINICAL TRIALS:			
02 BLADDER	07 CHEM.	2	8,183
04 BREAST	07 CHEM.	5	20,457
05 CERVIX	07 CHEM.	2	8,183
06 COLON	07 CHEM.	5	20,457
09 HEAD & NECK	07 CHEM.	2	8,183
11 KIDNEY	07 CHEM.	3	12,274
13 LUNG	07 CHEM.	5	20,457
15 MELANOMA	07 CHEM.	3	12,274
17 OVARY	07 CHEM.	5	20,457
18 PANCREAS	07 CHEM.	5	20,457
20 PROSTATE	07 CHEM.	5	20,457
21 SARCOMAS	07 CHEM.	5	20,457
22 STOMACH	07 CHEM.	3	12,274
		* 50*	204,570
PHASE III CLINICAL TRIALS:			
02 BLADDER	13 CHEM.	3	12,274
04 BREAST	13 CHEM.	5	20,457
05 CERVIX	13 CHEM.	3	12,274
06 COLON	13 CHEM.	5	20,457
09 HEAD & NECK	13 CHEM.	3	12,274
11 KIDNEY	13 CHEM.	2	8,183
13 LUNG	13 CHEM.	5	20,457
15 MELANOMA	13 CHEM.	*2	8,183
17 OVARY	13 CHEM.	5	20,457
18 PANCREAS	13 CHEM.	5	20,457
20 PROSTATE	13 CHEM.	5	20,457
21 SARCOMAS	13 CHEM.	5	20,457
22 STOMACH	13 CHEM.	2	8,183

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
MEMORIAL HOSP. FOR CANCER & ALLIED	N01CM57019			129,981
XXX PROGRAM MANAGEMENT:		* 10*	12,998	
01 ADMINISTRATION				
PHASE II CLINICAL TRIALS:		* 30*	38,995	
03 BRAIN	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 60*	77,988	
03 BRAIN	13 CHEM.	30	38,994	
03 BRAIN	15 RAD.	30	38,994	
MEMORIAL HOSP. FOR CANCER & ALLIED	N01CM43716			60,500
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		*100*	60,500	
23 TESTICULAR	20 CHEM.	34	20,570	
23 TESTICULAR	21 SURG.	33	19,965	
23 TESTICULAR	22 RAD.	33	19,965	
MIAMI, UNIVERSITY OF	N01CM57041			95,540
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 40*	38,216	
99 OTHER-IMMUNOLOGY				
2B2 VERIFICATION SCREEN:		* 60*	57,324	
01 DETAILED DRUG EVALUATION, IN VITRO		30	28,662	
02 DETAILED DRUG EVALUATION, IN VIVO		30	28,662	
MIAMI, UNIVERSITY OF	N01CM67077			124,953
1XX ACQUISITION OF MATERIALS:		* 5*	6,247	
05 BIOASSAY OF NATURAL PRODUCTS				
2A1 BASIC SCREEN:		* 95*	118,706	
01 PRIMARY SCREEN:ING, IN VITRO		90	112,458	
03 PRIMARY SCREEN:ING, REL. NEW MODEL DEVEL.		5	6,248	
MICHIGAN CANCER FOUNDATION	N01CM53789			53,671
2B2 VERIFICATION SCREEN:		*100*	53,671	
01 DETAILED DRUG EVALUATION, IN VITRO		80	42,937	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		20	10,734	
MICHIGAN DEPARTMENT OF PUBLIC HEALTH	N01CM33702			54,166
2A1 BASIC SCREEN:		*100*	54,166	
01 PRIMARY SCREEN:ING, IN VITRO				
MICROBIAL CHEMISTRY RESEARCH FDN.	N01CM57009			66,000
1XX ACQUISITION OF MATERIALS:		* 60*	39,600	
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
		* 40*	26,400	
2A1 BASIC SCREEN:				
03 PRIMARY SCREEN:ING, PEL. NEW MODEL DEVEL.				
				175,800
MICROBIOLOGICAL ASSOCIATES	N01CM67069			
		* 20*	35,160	
1XX ACQUISITION OF MATERIALS:				
06 ANIMALS		* 55*	96,690	
2A1 BASIC SCREEN:				
04 ANIMALS		* 25*	43,950	
2B2 VERIFICATION SCREEN:				
04 ANIMALS				
				2,596
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM92177			
		100	2,596	
PHASE III CLINICAL TRIALS:				
12 LEUKEMIA	18 TRANSP. RESEARCH			
				100,000
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM63810			
		100	100,000	
PHASE III CLINICAL TRIALS:				
12 LEUKEMIA	18 TRANSP. RESEARCH			
				435,800
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM43793			
		* 60*	261,480	
1XX ACQUISITION OF MATERIALS:		10	43,580	
98 DATA PROCESSING AND SUPPORT		50	217,900	
99 OTHER-STORAGE DISTRIBUTION		* 30*	130,740	
2B3 PROCUREMENT OF PRECLIN. MAT'L:				
99 OTHER-STORAGE DISTRIBUTION		* 10*	43,580	
3X3 PROD./FORM. FOR CLIN. TRIALS:		1	4,358	
98 DATA PROCESSING AND SUPPORT		9	39,222	
99 OTHER-STORAGE DISTRIBUTION				
				143,500
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM53818			
		* 20*	28,700	
1XX ACQUISITION OF MATERIALS:				
06 ANIMALS		* 55*	78,925	
2A1 BASIC SCREEN:				
04 ANIMALS		* 25*	35,875	
2B2 VERIFICATION SCREEN:				
04 ANIMALS				
				397,642
MICROBIOLOGICAL ASSOCIATES, INC.	N01CM43761			
		* 95*	377,760	
1XX ACQUISITION OF MATERIALS:				
01 NEW AGENT PROCUREMENT, SYNTHETICS		* 5*	19,882	
2B3 PROCUREMENT OF PRECLIN. MAT'L:				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
01 SYNTHETICS				
MIDWEST RESEARCH INSTITUTE	N01CM33722			217,958
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 20*	43,592	
06 ANALYTICAL AND QUALITY CONTROL				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 80*	174,366	
07 ANALYTICAL AND QUALITY CONTROL				
MIDWEST RESEARCH INSTITUTE	N01CM33743			375,630
1XX ACQUISITION OF MATERIALS:		* 80*	300,504	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2A1 BASIC SCREEN:		* 6*	22,538	
01 PRIMARY SCREENING, IN VITRO				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 14*	52,588	
01 SYNTHETICS				
MINNESOTA, UNIVERSITY OF	N01CM43788			70,801
2B2 VERIFICATION SCREEN:		*100*	70,801	
01 DETAILED DRUG EVALUATION, IN VITRO		80	56,641	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		20	14,160	
MISSOURI, UNIVERSITY OF	N01CM12323			173,000
PHASE II CLINICAL TRIALS:		*100*	173,000	
01 NONSPECIFIC	12 MARKERS			
MONSANTO RESEARCH CORPORATION	N01CM33709			1,208,879
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	120,888	
02 PLANT PRODUCTS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 90*	1,087,991	
01 PRODUCTION, SYNTHETICS		15	181,332	
02 PRODUCTION, PLANT PRODUCTS		75	906,659	
MONTEFIORE HOSPITAL	N01CM67061			56,274
XXX PROGRAM MANAGEMENT:		* 10*	5,627	
01 ADMINISTRATION				
PHASE II CLINICAL TRIALS:		* 30*	16,883	
03 BRAIN	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 60*	33,764	
03 BRAIN	13 CHEM.	30	16,882	
03 BRAIN	15 RAD.	30	16,882	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
01 NONSPECIFIC	11 PHARM./TOX.			
OHIO STATE UNIVERSITY	N01CM57015			64,327
1XX ACQUISITION OF MATERIALS:		* 90*	57,894	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	6,433	
01 SYNTHETICS				
OHIO STATE UNIVERSITY RESEARCH	N01CM67060			46,772
XXX PROGRAM MANAGEMENT:		* 10*	4,677	
01 ADMINISTRATION				
PHASE II CLINICAL TRIALS:		* 30*	14,031	
03 BRAIN	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 60*	28,064	
03 BRAIN	13 CHEM.	30	14,032	
03 BRAIN	15 RAD.	30	14,032	
OHIO STATE UNIVERSITY RESEARCH	N01CM33736			105,000
PHASE II CLINICAL TRIALS:		* 10*	10,500	
13 LUNG	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 60*	63,000	
13 LUNG	13 CHEM.	20	21,000	
13 LUNG	14 SURG.	20	21,000	
13 LUNG	15 RAD.	20	21,000	
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 30*	31,500	
13 LUNG	20 CHEM.	15	15,750	
13 LUNG	21 SURG.	15	15,750	
OREGON STATE UNIVERSITY	N01CM23201			93,840
3X1 PHARMACOLOGY/TOXICOLOGY:		*100*	93,840	
01 PHARMACOLOGY				
PARKE, DAVIS AND COMPANY	N01CM23708			405,646
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 50*	202,822	
01 SYNTHETICS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 50*	202,824	
01 PRODUCTION, SYNTHETICS		25	101,412	
02 PRODUCTION, PLANT PRODUCTS		25	101,412	
PARKE, DAVIS AND COMPANY	N01CM43754			746,081
1XX ACQUISITION OF MATERIALS:		* 85*	634,169	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS		83	619,247	
98 DATA PROCESSING AND SUPPORT		2	14,922	
2A1 BASIC SCREEN:		* 15*	111,912	
01 PRIMARY SCREENING, IN VITRO		10	74,608	
03 PRIMARY SCREENING, REL. NEW MODEL DEVEL.		5	37,304	
PENNSYLVANIA, UNIVERSITY OF	**N01CM33719**			**117,864**
1XX ACQUISITION OF MATERIALS:		*100*	117,864	
98 DATA PROCESSING AND SUPPORT				
PENNSYLVANIA, UNIVERSITY OF	**N01CM53830**			**22,656**
2B2 VERIFICATION SCREEN:		*100*	22,656	
01 DETAILED DRUG EVALUATION, IN VITRO		50	11,328	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		50	11,328	
PFIZER, INC.	**N01CM53805**			**359,861**
1XX ACQUISITION OF MATERIALS:		*100*	359,861	
99 OTHER-ANIMAL VIRUSES				
PHARM-ECO	**N01CM23707**			**180,607**
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 30*	54,182	
01 SYNTHETICS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 70*	126,425	
01 PRODUCTION, SYNTHETICS				
PHILIPS ROXANE LABORATORIES, INC.	**N01CM12139**			**40,000**
2B1 FORMULATION:		* 20*	8,000	
01 DEVEL. OF EXPERIMENTAL FORMULATIONS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 80*	32,000	
06 FORMULATION		70	28,000	
07 ANALYTICAL AND QUALITY CONTROL		10	4,000	
POMONA COLLEGE	**N01CM33703**			**43,800**
1XX ACQUISITION OF MATERIALS:		*100*	43,800	
99 OTHER-STRUCTURE ACTIVITY				
PUBLIC HEALTH SERVICE	**Y01CM40003**			**291,276**
3X1 PHARMACOLOGY/TOXICOLOGY:		* 30*	87,383	
01 PHARMACOLOGY				
XXX PROGRAM MANAGEMENT:		* 5*	14,564	
01 ADMINISTRATION				
PHASE I CLINICAL TRIALS:		* 35*	101,946	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
SOUTHERN ANIMAL FARMS	N01CM53845			134,660
1XX ACQUISITION OF MATERIALS:		* 20*	26,932	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	74,063	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	33,665	
04 ANIMALS				
SOUTHERN ANIMAL FARMS	N01CM50599			212,160
1XX ACQUISITION OF MATERIALS:		* 20*	42,432	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	116,688	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	53,040	
04 ANIMALS				
SOUTHERN RESEARCH INSTITUTE	N01CM43756			2,370,000
2A1 BASIC SCREEN:		* 32*	758,400	
01 PRIMARY SCREEN:ING, IN VITRO		6	142,200	
02 PRIMARY SCREEN:ING, IN VIVO		26	616,200	
2A2 BIOLOGICAL RESEARCH PROGRAMS:		* 5*	118,500	
02 CELL KINETICS				
2B2 VERIFICATION SCREEN:		* 48*	1,137,600	
01 DETAILED DRUG EVALUATION, IN VITRO		3	71,100	
02 DETAILED DRUG EVALUATION, IN VIVO		45	1,066,500	
3X2 PRECLIN. COMBINED TREATMENT:		* 15*	355,500	
01 COMBINATION CHEMOTHERAPY		11	260,700	
02 COMBINED MODALITY PROGRAM		4	94,800	
SOUTHERN RESEARCH INSTITUTE	N01CM43784			400,804
2B2 VERIFICATION SCREEN:		*100*	400,804	
01 DETAILED DRUG EVALUATION, IN VITRO		60	240,482	
02 DETAILED DRUG EVALUATION, IN VIVO		20	80,161	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		20	80,161	
SOUTHERN RESEARCH INSTITUTE	N01CM43780			457,008
3X1 PHARMACOLOGY/TOXICOLOGY:		*100*	457,008	
01 PHARMACOLOGY				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
SOUTHERN RESEARCH INSTITUTE	N01CM43762			659,728
1XX ACQUISITION OF MATERIALS:		* 80*	527,782	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 20*	131,946	
01 SYNTHETICS				
SOUTHERN RESEARCH INSTITUTE	N01CM57000			360,545
3X1 PHARMACOLOGY/TOXICOLOGY:		* 70*	252,381	
03 SPECIAL TOXICITY STUDIES				
3X2 PRECLIN. COMBINED TREATMENT:		* 30*	108,164	
01 COMBINATION CHEMOTHERAPY				
SOUTHWEST FOUNDATION FOR RESEARCH &	N01CM67075			97,721
1XX ACQUISITION OF MATERIALS:		* 5*	4,886	
05 BIOASSAY OF NATURAL PRODUCTS				
2A1 BASIC SCREEN:		* 95*	92,835	
01 PRIMARY SCREEN:ING, IN VITRO		90	87,949	
03 PRIMARY SCREEN:ING, REL. NEW MODEL DEVEL.		5	4,886	
SPARTAN RESEARCH ANIMALS, INC.	N01CM50582			106,080
1XX ACQUISITION OF MATERIALS:		* 20*	21,216	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	58,344	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	26,520	
04 ANIMALS				
ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020			112,832
XXX PROGRAM MANAGEMENT:		* 10*	11,283	
01 ADMINISTRATION				
PHASE II CLINICAL TRIALS:		* 30*	33,849	
03 BRAIN 07 CHEM.				
PHASE III CLINICAL TRIALS:		* 60*	67,700	
03 BRAIN 13 CHEM.		30	33,850	
03 BRAIN 15 RAD.		30	33,850	
STANFORD RESEARCH INSTITUTE	N01CM33723			345,789
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 20*	69,158	
06 ANALYTICAL AND QUALITY CONTROL				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 80*	276,631	
07 ANALYTICAL AND QUALITY CONTROL				

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
STANFORD RESEARCH INSTITUTE	N01CM33742			475,240
1XX ACQUISITION OF MATERIALS:		* 75*	356,430	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2A1 BASIC SCREEN:		* 4*	19,010	
01 PRIMARY SCREEN:ING, IN VITRO				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 21*	99,800	
01 SYNTHETICS				
STARKS ASSOCIATES, INC.	N01CM23203			392,576
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 50*	196,288	
01 SYNTHETICS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 50*	196,288	
01 PRODUCTION, SYNTHETICS				
STARKS ASSOCIATES, INC.	N01CM53769			167,090
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 75*	125,317	
01 SYNTHETICS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 25*	41,773	
01 PRODUCTION, SYNTHETICS				
STARKS, C. P., INC.	N01CM53745			373,263
1XX ACQUISITION OF MATERIALS:		*100*	373,263	
01 NEW AGENT PROCUREMENT, SYNTHETICS		70	261,284	
98 DATA PROCESSING AND SUPPORT		30	111,979	
TACONIC FARMS	N01CM50597			171,600
1XX ACQUISITION OF MATERIALS:		* 20*	34,320	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	94,380	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	42,900	
04 ANIMALS				
TENNESSEE, UNIVERSITY OF	N01CM02084			20,000
2B1 FORMULATION:		* 25*	5,000	
01 DEVEL. OF EXPERIMENTAL FORMULATIONS				
3X3 PROD./FORM. FOR CLIN. TRIALS:		* 75*	15,000	
06 FORMULATION		65	13,000	
07 ANALYTICAL AND QUALITY CONTROL		10	2,000	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
TENNESSEE, UNIVERSITY OF	N01CM57021			99,717
XXX PROGRAM MANAGEMENT:		* 10*	9,972	
01 ADMINISTRATION				
PHASE II CLINICAL TRIALS:		* 30*	29,915	
03 BRAIN	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 60*	59,830	
03 BRAIN	13 CHEM.	30	29,915	
03 BRAIN	15 RAD.	30	29,915	
TEXAS INBRED MICE	N01CM53816			473,275
1XX ACQUISITION OF MATERIALS:		* 20*	94,655	
06 ANIMALS				
2A1 BASIC SCREEN:		* 55*	260,301	
04 ANIMALS				
2B2 VERIFICATION SCREEN:		* 25*	118,319	
04 ANIMALS				
TEXAS, UNIV. OF-M.D. ANDERSON	N01CM53773			486,063
3X1 PHARMACOLOGY/TOXICOLOGY:		* 60*	291,638	
01 PHARMACOLOGY				
PHASE I CLINICAL TRIALS:		* 40*	194,425	
01 NONSPECIFIC	05 PHARM./TOX.			
TEXAS, UNIVERSITY OF-SYSTEM CANCER	N01CM57042			434,189
PHASE II CLINICAL TRIALS:		* 50*	217,096	
02 BLADDER	07 CHEM.	2	8,684	
04 BREAST	07 CHEM.	5	21,709	
05 CERVIX	07 CHEM.	2	8,684	
06 COLON	07 CHEM.	5	21,709	
09 HEAD & NECK	07 CHEM.	2	8,684	
11 KIDNEY	07 CHEM.	3	13,026	
13 LUNG	07 CHEM.	5	21,709	
15 MELANOMA	07 CHEM.	3	13,026	
17 OVARY	07 CHEM.	5	21,709	
18 PANCREAS	07 CHEM.	5	21,709	
20 PROSTATE	07 CHEM.	5	21,709	
21 SARCOMAS	07 CHEM.	5	21,709	
22 STOMACH	07 CHEM.	3	13,026	
PHASE III CLINICAL TRIALS:		* 50*	217,093	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
02 BLADDER	13 CHEM.	3	13,026	
04 BREAST	13 CHEM.	5	21,709	
05 CERVIX	13 CHEM.	3	13,026	
06 COLON	13 CHEM.	5	21,709	
09 HEAD & NECK	13 CHEM.	3	13,026	
11 KIDNEY	13 CHEM.	2	8,684	
13 LUNG	13 CHEM.	5	21,709	
15 MELANOMA	13 CHEM.	2	8,684	
17 OVARY	13 CHEM.	5	21,709	
18 PANCREAS	13 CHEM.	5	21,709	
20 PROSTATE	13 CHEM.	5	21,709	
21 SARCOMAS	13 CHEM.	5	21,709	
22 STOMACH	13 CHEM.	2	8,684	
TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM33737			413,700
PHASE II CLINICAL TRIALS:		* 10*	41,370	
13 LUNG	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 60*	248,220	
13 LUNG	13 CHEM.	20	82,740	
13 LUNG	14 SURG.	20	82,740	
13 LUNG	15 RAD.	20	82,740	
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 30*	124,110	
13 LUNG	20 CHEM.	15	62,055	
13 LUNG	21 SURG.	15	62,055	
TEXAS, UNIVERSITY OF, M.D. ANDERSON	N01CM33710			200,000
PHASE II CLINICAL TRIALS:		* 10*	20,000	
17 OVARY	07 CHEM.			
PHASE III CLINICAL TRIALS:		* 70*	140,000	
17 OVARY	13 CHEM.			
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 20*	40,000	
17 OVARY	20 CHEM.			
TEXAS, UNIVERSITY OF, SYSTEM CANCER	N01CM43786			85,550
2B2 VERIFICATION SCREEN:		*100*	85,550	
01 DETAILED DRUG EVALUATION, IN VITRO		60	51,330	
02 DETAILED DRUG EVALUATION IN VIVO		20	17,110	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		20	17,110	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT	DOLLAR LEVEL
TEXAS, UNIVERSITY OF	N01CM53767		66,773
2B2 VERIFICATION SCREEN:		*100*	66,773
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.			
TEXAS, UNIVERSITY OF	N01CM53832		605,000
PHASE III CLINICAL TRIALS:		*100*	605,000
14 LYMPHOMAS	19 PROTECT. ENV.	50	302,500
21 SARCOMAS	19 PROTECT. ENV.	50	302,500
TULANE UNIVERSITY	N01CM53815		195,504
1XX ACQUISITION OF MATERIALS:		* 20*	39,101
06 ANIMALS			
2A1 BASIC SCREEN:		* 55*	107,527
04 ANIMALS			
2B2 VERIFICATION SCREEN:		* 25*	48,876
04 ANIMALS			
UPJOHN COMPANY	N01CM43753		1,200,480
1XX ACQUISITION OF MATERIALS:		* 57*	684,273
03 NEW AGENT PROCURE., FERMENT./ANTIBIOTICS		55	660,264
98 DATA PROCESSING AND SUPPORT		2	24,010
2A1 BASIC SCREEN:		* 17*	204,082
01 PRIMARY SCREEN:ING, IN VITRO		7	84,034
02 PRIMARY SCREEN:ING, IN VIVO		3	36,014
03 PRIMARY SCREEN:ING, REL. NEW MODEL DEVEL.		7	84,034
2B1 FORMULATION:		* 1*	12,005
01 DEVEL. OF EXPERIMENTAL FORMULATIONS			
2B2 VERIFICATION SCREEN:		* 10*	120,048
99 OTHER-MOLECULAR BIOLOGY			
3X1 PHARMACOLOGY/TOXICOLOGY:		* 15*	180,072
01 PHARMACOLOGY		13	156,062
98 DATA PROCESSING AND SUPPORT		2	24,010
UTAH, UNIVERSITY OF	N01CM53827		62,362
3X1 PHARMACOLOGY/TOXICOLOGY:		*100*	62,362
03 SPECIAL TOXICITY STUDIES			
UTAH, UNIVERSITY OF	N01CM43806		225,256
1XX ACQUISITION OF MATERIALS:		* 70*	157,679
01 NEW AGENT PROCUREMENT, SYNTHETICS			

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
2A1 BASIC SCREEN:		* 10*	22,526	
01 PRIMARY SCREEN:ING, IN VITRO				
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 20*	45,051	
01 SYNTHETICS				
VALUE ENGINEERING COMPANY	N01CM53821			105,978
XXX PROGRAM MANAGEMENT:		*100*	105,978	
98 DATA PROCESSING AND SUPPORT				
VALUE ENGINEERING COMPANY	N01CM33706			898,724
1XX ACQUISITION OF MATERIALS:		* 14*	125,821	
05 BIOASSAY OF NATURAL PRODUCTS				
2A1 BASIC SCREEN:		* 58*	521,260	
01 PRIMARY SCREEN:ING, IN VITRO		5	44,936	
02 PRIMARY SCREEN:ING, IN VIVO		53	476,324	
2B2 VERIFICATION SCREEN:		* 20*	179,745	
02 DETAILED DRUG EVALUATION, IN VIVO				
3X2 PRECLIN. COMBINED TREATMENT:		* 8*	71,898	
01 COMBINATION CHEMOTHERAPY				
VERMONT, UNIVERSITY OF	N01CM33716			33,843
XXX PROGRAM MANAGEMENT:		* 20*	6,768	
01 ADMINISTRATION		1	338	
02 COMMUNICATION AND EDUCATION		9	3,046	
98 DATA PROCESSING AND SUPPORT		10	3,384	
PHASE III CLINICAL TRIALS:		* 80*	27,075	
01 NONSPECIFIC	13 CHEM.	30	10,153	
12 LEUKEMIA	19 PROJECT. ENV.	50	16,922	
VETERANS ADMINISTRATION	Y01CM60103			554
3X1 PHARMACOLOGY/TOXICOLOGY:		*100*	554	
03 SPECIAL TOXICITY STUDIES				
VETERANS ADMINISTRATION	Y01CM40004			3,532,909
PHASE I CLINICAL TRIALS:		* 10*	353,293	
01 NONSPECIFIC	01 CHEM.			
PHASE II CLINICAL TRIALS:		* 20*	706,581	
06 COLON	07 CHEM.	1	35,329	
13 LUNG	07 CHEM.	15	529,936	
18 PANCREAS	07 CHEM.	1	35,329	

CONTRACTOR/FUNCTIONS		PERCENT OF EFFORT	DOLLAR LEVEL
20 PROSTATE	07 CHEM.	2	70,658
22 STOMACH	07 CHEM.	1	35,329
PHASE III CLINICAL TRIALS:		* 50*	1,766,453
06 COLON	13 CHEM.	3	105,987
13 LUNG	13 CHEM.	20	706,582
13 LUNG	15 RAD.	15	529,936
18 PANCREAS	13 CHEM.	4	141,316
20 PROSTATE	13 CHEM.	5	176,645
22 STOMACH	13 CHEM.	3	105,987
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 20*	706,582
06 COLON	20 CHEM.	1	35,329
06 COLON	21 SURG.	1	35,329
13 LUNG	20 CHEM.	7	247,304
13 LUNG	21 SURG.	8	282,633
18 PANCREAS	20 CHEM.	1	35,329
18 PANCREAS	21 SURG.	1	35,329
22 STOMACH	20 CHEM.	1	35,329
VETERANS ADMINISTRATION	**Y01CM60102**		**45,472**
1XX ACQUISITION OF MATERIALS:		* 90*	40,925
01 NEW AGENT PROCUREMENT, SYNTHETICS			
2B3 PROCUREMENT OF PRECLIN. MAT'L:		* 10*	4,547
01 SYNTHETICS			
WALTER REED ARMY MEDICAL CENTER	**Y01CM40005**		**30,000**
PHASE I CLINICAL TRIALS:		* 8*	2,400
04 BREAST	01 CHEM.		
PHASE II CLINICAL TRIALS:		* 38*	11,400
04 BREAST	07 CHEM.	34	10,200
04 BREAST	12 MARKERS	4	1,200
PHASE III CLINICAL TRIALS:		* 33*	9,900
04 BREAST	13 CHEM.	13	3,900
12 LEUKEMIA	13 CHEM.	5	1,500
14 LYMPHOMAS	13 CHEM.	8	2,400
14 LYMPHOMAS	15 RAD.	2	600
23 TESTICULAR	13 CHEM.	5	1,500
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 13*	3,900

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
04 BREAST	20 CHEM.	1	300	
04 BREAST	21 SURG.	1	300	
04 BREAST	22 RAD.	1	300	
04 BREAST	23 IMM.	1	300	
13 LUNG	20 CHEM.	1	300	
13 LUNG	21 SURG.	1	300	
13 LUNG	22 RAD.	1	300	
13 LUNG	23 IMM.	1	300	
14 LYMPHOMAS	20 CHEM.	1	300	
14 LYMPHOMAS	22 RAD.	1	300	
23 TESTICULAR	20 CHEM.	1	300	
23 TESTICULAR	21 SURG.	1	300	
23 TESTICULAR	22 RAD.	1	300	
D.O.S. DATA PROCESSING:		* 8*	2,400	
01 NONSPECIFIC	25 DATA PROCESSING			
WARF INSTITUTE, INC.	N01CM43707			111,287
1XX ACQUISITION OF MATERIALS:		*100*	111,287	
02 NEW AGENT PROCUREMENT, PLANT PRODUCTS				
WASHINGTON UNIVERSITY SCHOOL O	N01CM43731			112,243
2B2 VERIFICATION SCREEN:		* 80*	89,794	
01 DETAILED DRUG EVALUATION, IN VITRO		15	16,836	
02 DETAILED DRUG EVALUATION, IN VIVO		65	72,958	
3X2 PRECLIN. COMBINED TREATMENT:		* 20*	22,449	
02 COMBINED MODALITY PROGRAM				
WISCONSIN, UNIVERSITY OF	N01CM12275			95,000
2A2 BIOLOGICAL RESEARCH PROGRAMS:		*100*	95,000	
01 MOLECULAR BIOLOGY				
WISCONSIN, UNIVERSITY OF	N01CM33733			685
1XX ACQUISITION OF MATERIALS:		* 35*	240	
05 BIOASSAY OF NATURAL PRODUCTS				
2A1 BASIC SCREEN:		* 65*	445	
01 PRIMARY SCREENING, IN VITRO		60	411	
03 PRIMARY SCREENING, REL. NEW MODEL DEVEL.		5	34	
WISCONSIN, UNIVERSITY OF	N01CM67076			56,262
1XX ACQUISITION OF MATERIALS:		* 5*	2,813	

CONTRACTOR/FUNCTIONS	#	PERCENT OF EFFORT		DOLLAR LEVEL
05 BIOASSAY OF NATUPAL PRODUCTS				
2A1 BASIC SCPEEN:		* 95*	53,449	
01 PRIMARY SCREEN:ING, IN VITRO		90	50,636	
03 PRIMAPY SCREEN:ING, REL. NEW MODEL DEVEL.		5	2,813	
YALE UNIVERSITY	N01CM57029			90,421
1XX ACQUISITION OF MATERIALS:		* 75*	67,815	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2A1 BASIC SCREEN:		* 25*	22,606	
01 PRIMARY SCREEN:ING, IN VITRO		12	10,851	
02 PRIMARY SCREEN:ING, IN VIVO		13	11,755	
YALE UNIVERSITY	N01CM53843			107,310
PHASE II CLINICAL TRIALS:		* 20*	21,462	
22 STOMACH	07 CHEM.			
PHASE IV CLINICAL TRIALS (COMB. MODAL.):		* 80*	85,848	
22 STOMACH	20 CHEM.	30	32,193	
22 STOMACH	21 SURG.	30	32,193	
22 STOMACH	22 RAD.	20	21,462	
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM53824			81,039
1XX ACQUISITION OF MATERIALS:		* 67*	54,296	
01 NEW AGENT PROCUREMENT, SYNTHETICS				
2A1 BASIC SCREEN:		* 33*	26,743	
01 PRIMARY SCREEN:ING, IN VITRO		30	24,312	
02 PRIMARY SCREEN:ING, IN VIVO		3	2,431	
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM33711			21,000
2A1 BASIC SCREEN:		* 30*	6,300	
01 PRIMARY SCREEN:ING, IN VITRO				
3X1 PHARMACOLOGY/TOXICOLOGY:		* 35*	7,350	
01 PHARMACOLOGY		20	4,200	
99 OTHER-PHARMACOKINETICS		15	3,150	
PHASE I CLINICAL TRIALS:		* 35*	7,350	
01 NONSPECIFIC	05 PHARM./TOX.			
YESHIVA UNIVERSITY	N01CM43787			91,632
2B2 VERIFICATION SCREEN:		*100*	91,632	
01 DETAILED DRUG EVALUATION, IN VITRO		80	73,306	
03 DETAILED DRUG EVAL., REL. NEW MODEL DEV.		20	18,326	

TABLE IV
DESCRIPTION OF CONTRACTS
IN THE
DIVISION OF CANCER TREATMENT PROGRAM

ABBOTT LABORATORIES (NO1-CM4-3704)

This contract was designed to develop a conditioning medium that will cause normal and leukemic granulocytic precursor cells to differentiate and form colonies of mature granulocytes. The Contractor supplies large amounts of active, purified conditioning factor that will be considered acceptable for clinical trials. The material will have potential clinical use in leukemia, aplastic anemia and drug-induced aplasias.

AGRICULTURE, U. S. DEPARTMENT OF (TRANSFER OF FUNDS) (YO1-CM4-0001)

Under the Transfer of Funds Agreement with the Medicinal Plant Resources Laboratory of the U.S.D.A., worldwide general plant collections and recollections are made. The general plant collections are shipped to WARF Institute, Inc. for extraction and the extracts are screened in vitro and in vivo for antineoplastic activity. Confirmed active plants are recollected in large quantities on a priority based on the needs of the Division of Cancer Treatment Program for shipment to the fractionating chemists. Very large recollections are made, as requested by the Drug Research and Development Program, for the preparation of clinical quantities of drugs. Large collections are made of species, not previously screened, that are members of the families known to be rich sources of activity. The nomenclature of all plants acquisitioned is being continuously reviewed and corrected, and a computer file of all plants is being maintained.

ALBANY MEDICAL COLLEGE (NO1-CM5-7032)

This contract is designed to support prospective, randomized controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

ALDRICH CHEMICAL COMPANY (NO1-CM2-3706)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 30% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

ALLEGHENY GENERAL HOSPITAL (NO1-CM4-3730)

The objective of this contract is to determine the dose and time course effects of antitumor drugs selected by the National Cancer Institute on survival of normal gastrointestinal crypts and on the in vivo survival of experimental murine mastocytoma cells. The effects of multiple drug dose interval on the normal gastrointestinal mucosa and the mastocytoma (in various metastatic sites) will be evaluated. Information on dose schedules and drug combinations leading to minimal or maximal gastrointestinal toxicity will be provided concurrent with the effects of these therapies on a proliferating murine mastocytoma. The methods used in these studies to determine gastrointestinal crypt survival were developed by the Contractor.

ARIZONA BOARD OF REGENTS (ARIZONA STATE UNIVERSITY) (NO1-CM1-2308)

The major objective of this contract is the preparation, from animal, insect and plant materials including marine species, of extracts suitable for bioassay in the National Cancer Institute's antitumor screen. The raw natural product specimens are obtained in most instances by the Contractor. The extracts are prepared in accordance with procedures acceptable to the National Cancer Institute and in amounts suitable for routine in vivo screening. Extracts showing preliminary activity are prepared anew for confirmatory testing and materials showing confirmed activity are assigned for fractionation and isolation. This contract is being recompeted this year.

ARIZONA BOARD OF REGENTS (UNIVERSITY OF ARIZONA) (NO1-CM3-3750)

The major objective of this contract is the fractionation of confirmed active plants in order to isolate in a pure state and identify the antitumor active components. A KB cell culture unit has been added to expedite the fractionation work. As a result of a recompetition, this will be the terminal year for this contract.

ARIZONA, UNIVERSITY OF (NO1-CM3-3713)

This contract is devoted to the further development of a staging system for patients with multiple myeloma. The rationale for this proposed staging system was based on detailed studies by the Principal Investigator, Dr. Sydney Salmon, in working out a procedure for determining total myeloma cell number in patients. The proposed system incorporates available clinical and laboratory data and measurements in conjunction with tumor cell number to derive a means for staging myeloma patients. This system is now being tested prospectively to determine if the desired correlations with actual tumor cell measurements hold true. It is anticipated this system could be used for evaluation of the number of tumor cells remaining following therapy using clinical parameters without the necessity for carrying out the detailed and involved procedures for actual measurement of this number.

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE (YO1-CM6-0104)

The funds for the initial year were to be used by the Contractor to investig whether AFRRI would be capable of animal studies aimed at the study of metho to treat radiation-induced bone marrow failure. There has been no formal progress report received to date, but the Contractor has been advised that t effort is to be discontinued because of a change in emphasis in the Pediatri Oncology Branch program.

ARS/SPRAGUE-DAWLEY (NO1-CM5-3813)

This primary genetic center produces a variety of outbred, inbred and hybrid of inbred rodents. All production activities are effected in closely controlled environments. The overall workscope comprises four distinct task identified as follows:

Task 1 provides inbred, outbred and hybrid rodents in modified conventional environments.
Task 2 provides Syrian golden hamsters with defined but non-pathogenic organ A small percentage of the effort is devoted to the development of germ-free hamsters.
Task 3 provides inbred and outbred laboratory strains of rats as germ-free and/or with defined microflora.
Task 4 provides various germ-free inbred, outbred and hybrids of inbred mice and the production of counterparts of the various species with defined micro flora.

ARS/SPRAGUE-DAWLEY (NO1-CM5-3846)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlat with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. Major emphasis is upo the production of AKR DBA/2 and C57BL/6 mice.

ARTHUR D. LITTLE, INC. (NO1-CM3-3727)

All segments of this formerly multifaceted contract have been either recompe terminated or effected as separate contracts with the exception of the Appli Mathematics and Data Analysis Project and the Central Nervous System Solid Tumor Studies.

The Applied Mathematics and Data Analysis Project supplies biostatistical support to the Drug Evaluation Branch, Drug Research and Development Program Division of Cancer Treatment, specifically to aid in establishing parameters and criteria of activity and toxicity in current and new antitumor screens, in formulating detailed experimental protocols for use in contract testing laboratories, and in maintaining testing quality control by evaluating screening methodology with respect to test-to-test and lab-to-lab variabilit

ARTHUR D. LITTLE, INC. (NO1-CM3-3727) (CONTINUED)

The workscope includes studies of individual screening models (animal tumor assays) with regard to their validity (e.g., reproducibility) and their yield of active materials and to determine the degree of correlations among alternative models. Additionally, this project includes tasks related to the evaluation and summarization of clinical results. Analysis of data from antitumor screening laboratories and from the clinic as well provides for the quantitative assessment of correlations between activity against specific laboratory models and activity against specific human malignancies.

During the period of this Annual Report, the contract also included continuation of the Central Nervous System Solid Tumor Studies, pending completion of action to award a separate contract. These studies were concerned with the development and evaluation of transplantable gliomas in the CNS of beagle dogs, together with collaborative studies with clinical and pharmacological groups of the National Cancer Institute to examine the growth kinetics and the behavior of transplantable gliomas to selected groups.

ARTHUR D. LITTLE, INC. (NO1-CM5-3765)

The three principal facets of this project are: (1) in vivo antitumor screening of structural congeners, "analogs", of known antitumor drugs to aid in assessing the potential superiority of an analog over the known parent compound and to supply results of testing directly and rapidly to organic chemists to aid the direction of new agent synthesis. The test systems used are designed specifically for each compound class of interest; (2) studies of combinations of drugs to determine their potential superiority over the individual drugs; and (3) studies of the effects of radiation therapy and combinations of drugs plus radiation. Considerable emphasis is placed on the quantitative comparative evaluation of potential radiation-sensitizing chemical agents.

ARTHUR D. LITTLE, INC. (NO1-CM5-3849)

This contract encompasses three distinct work areas: (1) physiological disposition and biochemical pharmacology, (2) electron microscopy and radioautography, and (3) tumor bank distribution services. Under the first activity work is conducted on the physiological disposition and biochemical pharmacology of antitumor agents under active development in the Division of Cancer Treatment Program. Work has been conducted during the past year on ellipticine, dichloroallyl lawsone, 5-azacytidine and adriamycin. Techniques for studying drug metabolism using isolated hepatocytes have been also developed. Electron microscopy and radioautography have focused on the tissue localization of melphalan, ara-C, fluorocyclocytidine, a Cain acridine, NSC-141549, and the cytopathology of the cerebellum and parotid salivary glands after ellipticine and cyclocytidine respectively. Finally, approximately 410 tumor sublines have been shipped to screening laboratories and investigators around the globe and a bank of almost 750 tumor sublines are maintained continuously.

ARTHUR D. LITTLE, INC. (NO1-CM5-7006)

This is a service contract for the in vivo screening of new synthetic and natural product materials and the follow-up testing of active materials. The primary screening is carried out in accordance with Drug Research and Development Program protocols which require new materials to be tested against the P388 leukemia test system. The current level of testing is approximately 25,000 L1210 equivalent tests per year. As requested, detailed testing is performed with other tumor test systems, such as the L1210 leukemia, the Lewis lung carcinoma and the B16 melanoma, and schedule dependency studies conducted with materials of potential clinical interest. Methodology studies, such as the characterization and evaluation of tumor test systems, are carried out as directed by the Project Officer.

ARTHUR D. LITTLE, INC. (NO1-CM6-3823) (FORMERLY NO1-CM2-3711)

This contract provides broad biomedical engineering resources which are responsive to the support of individual research projects within the Divisi of Cancer Treatment. A flexible mechanism of support defines and implement engineering projects in close collaboration with the Division of Cancer Treatment staff in active areas such as: automated microspectrometry, cell kinetics modeling, the automation of grain counting and bone marrow colony assays and studies of the protective laminar flow environment for supportiv care of leukemic patients. A "core group" of senior engineers continually consults and interacts with the National Cancer Institute staff on the definition of problems and projects while "project groups" are tailored to implement specific engineering tasks under National Cancer Institute staff direction.

ARTHUR D. LITTLE, INC. (NO1-CM6-7047)

The workscope of this program is to extend for a three-year period the research objectives of the Central Nervous System Solid Tumor Studies with the neonate-puppy animal model. This will include the transplantation of gliomas in the CNS of neonate beagle puppies to provide suitable tumor material for chemotherapy studies; chemotherapy studies with selected agent against the transplantable gliosarcoma in the brains of newborn puppies; histopathologic evaluation of the effects of chemotherapeutic agents agains the transplantable gliosarcoma in the brains of newborn puppies; collaborat studies with clinical and pharmacology groups of the National Cancer Instit to study the growth kinetics and biologic behavior of transplantable glioma to selected agents; methodological studies as agreed upon with the approval of the Project Officer and exploring the use of the transplantable beagle tumor in heterologous hosts such as rodents (rats, hamsters).

ARTHUR D. LITTLE, INC. (NO1-CM6-7078)

The multifacet Arthur D. Little contract (NO1-CM3-3727) was competed by segment and this contract is the result of a competitive competition for

ARTHUR D. LITTLE, INC. (NO1-CM6-7078) (CONTINUED)

tissue culture screening. Testing capacity is at the level of 10,000 KB test equivalents. Synthetic materials in too small a quantity for in vivo testing, crude natural products, and fractions of confirmed active natural products are tested in either the 9KB, L1210 lymphocytic leukemia, or the P388 lymphoid leukemias. The Drug Research and Development Program has proposed the addition of the Friend virus erythroid leukemia system to the in vitro tumor system program. Prior to the testing of all synthetic admissions in this tumor model, this laboratory is conducting studies leading to the development of a protocol that can be followed by other in vitro laboratories. Research and development are conducted at the request of the Drug Evaluation Branch.

ASH STEVENS, INC. (NO1-CM5-7012)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts required. About 75% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

ASSOCIATED BIOMEDIC SYSTEMS (NO1-CM0-2271)

The purposes of this contract are: (1) to supply normal human blood lymphocytes, both untreated and stimulated with phytohemagglutinin; (2) to produce and evaluate human myeloid colony-stimulating factor(s) by "conditioning" media with various cells and then testing such media for "factor" activity; (3) to perform various services regarding culture of human cell lines, such as (a) mass production of "interesting" lines (e.g., cells containing RNA-directed DNA polymerase, DNA polymerase γ, terminal deoxynucleotidyl transferase, or cell-producing "factors"), and (b) ridding cell lines of contaminating mycoplasma by various techniques. About 250 billion lymphocytes were provided last year. Also, 500 billion cultured lymphoblastoid cells were provided. Certain cell-free "conditioned" media have been identified as rich sources of colony-stimulating factor. At least one cell line has been freed of detectable mycoplasma.

AUTOMATION INDUSTRIES, INC. (NO1-CM3-3717)

This contract provides support for the Clinical Oncology area in the collection and computer processing of clinical data. The Contractor extracts data from medical records and other clinical sources and assists in the development and maintenance of computer files, and the generation of computer retrievals and reports. During the past year, the Contractor has demonstrated excellent capabilities in the accurate extraction and computerization of medical data.

BATTELLE MEMORIAL INSTITUTE (NO1-CM4-3746)

In March 1974, a prime contract was awarded with Battelle Memorial Laborato for supervision of all contracts carrying out the toxicologic evaluation of potential oncolytic agents under the aegis of the Laboratory of Toxicology. Through the prime contract mechanism, eight contracts formerly carrying out these studies have been consolidated under a single management-type contrac The workscope is comprised of four tasks as follows: Task I - Full Protoco Studies; Task II - High Priority Toxicity Studies; Task III - Specific Orga Testing; and Task IV - Automation of Toxicity Data.

BATTELLE MEMORIAL INSTITUTE (NO1-CM5-7005)

Battelle Columbus Laboratories conducts in vivo testing of new synthetic materials and natural products, of both plant and animal origin, in additio to the secondary evaluation of active materials of interest to the Drug Research and Development Program. This testing is conducted at a level of effort equivalent to approximately 50,000 leukemia L1210 tests per annum. Testing is conducted according to current Drug Research and Development Program protocols, i.e., new synthetic materials and new natural products are tested in the P388 leukemia. When requested, Battelle Columbus schedul the secondary evaluation of materials of Drug Research and Development Program interest in the B16 melanoma and Lewis lung carcinoma. Schedule dependency studies are also conducted with materials of potential clinical interest. Tumors are maintained in a tumor bank and are available to the Drug Research and Development Program. The Project Officer, dependent upon the current needs of the Drug Research and Development Program, may change the tumor assay systems. Current methodology and research is being conduct in the development and use of human xenografts in nude mice.

BAYLOR COLLEGE OF MEDICINE (NO1-CM5-7018)

The purpose of this contract is to evaluate the usefulness of the hyperplas nodule system (HAN) in the selection of potential anticancer agents for the clinic. Hyperplastic nodules are mammary alveolar tissue from female mice which is altered from normal but is distinct from malignant tissue. These nodules grow in cleared mammary fat pads and may be carried indefinitely by transplant. A number of stable lines have been established, from each of which mammary tumors arise over a characteristic time interval and grow in a uniform manner. The HAN system provides reproducible spontaneous mammary tumors for testing a limited number of drugs. The effects on three parameters are possible: (1) on the growth of the hyperplastic nodule transplant in the fat pad, (2) on numbers and time of appearance of tumors, and (3) on tumor growth.

BELLAIRE ACRES (NIH-75-C-583)

This contract furnishes approximately 312,000 CD2F_1 (BALB/c♀ x DBA/2♂) hybr mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

BEN VENUE LABORATORIES, INC. (NO1-CM5-3811)

This contract is a service contract to provide for the development and production of parenteral clinical dosage forms of antitumor agents. Ben Venue Laboratories, Inc. has the capacity for preparing large-scale production batches of dry-filled, liquid-filled and lyophilized sterile products. Specifically, the Contractor performs the following duties: (1) formulation development of sterile parenteral products, (2) production and quality assurance testing of parenteral dosage forms for clinical use, and (3) stability surveillance of all dosage forms produced. All products produced are packaged, labelled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

BOWMAN GRAY SCHOOL OF MEDICINE (NO1-CM6-7054) (FORMERLY NO1-CM8-0950)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

BRIGHAM YOUNG UNIVERSITY (NO1-CM4-3790)

The major emphasis of this project is the detailed evaluation of selected new antitumor drugs on folic acid metabolism. In addition to a study of drug action on thymidylate synthetase and dihydrofolate reductase, drug effects on serine transhydroxymethylase, N^5, N^{10}-methylene THF reductase, N^5-methyl THF homocysteine transmethylase, N^5, N^{10}-methylene THF dehydrogenase, N^5, N^{10}-methenyl THF cyclohydrase, 5-amino,4-imidazolecarboxamide ribonucleotide transformylase and glycinamide ribonucleotide transformylase will be studied.

BRISTOL-MYERS COMPANY (NO1-CM3-3751)

This is a no-cost contract concerned with the marketing of the clinically useful nitrosourea, BCNU. The Contractor summarizes the clinical and other data, prepares and files the New Drug Application (NDA) with the Food and Drug Administration, and continues to prosecute the NDA until it is approved. Once the NDA is approved, the Contractor will arrange for appropriate marketing and other aspects of assuring supplies of the drugs to the public. Approval of the NDA is expected shortly.

BRISTOL-MYERS COMPANY (NO1-CM4-3759)

The major objective of this fermentation contract is the preparation of novel antibiotics. These new antibiotics are obtained using the fermentation techniques of biotransformation and co-metabolism of antibiotics, chemicals

BRISTOL-MYERS COMPANY (NO1-CM4-3759) (CONTINUED)

and plant materials. This effort is to convert materials which have solubil toxic or marginal activity problems, into worthwhile agents. A multitude o unique organisms are isolated and used to obtain these microbial conversions in an effort to obtain new antineoplastic agents.

BRISTOL-MYERS COMPANY (NO1-CM6-7051)

This is a no-cost contract concerned with the marketing of the clinically useful nitrosourea, CCNU. The Contractor summarizes the clinical and other data, prepares and files the New Drug Application (NDA) with the Food and Drug Administration, and continues to prosecute the NDA until it is approve Once the NDA is approved, the Contractor will arrange for appropriate marke and other aspects of assuring supplies of the drugs to the public. Approva of the NDA is expected shortly.

BRITISH COLUMBIA, UNIVERSITY OF (NO1-CM2-3223)

The major objective of this contract is to develop a general and versatile synthesis of the highly complex and clinically effective dimeric vinca alkaloids, vincristine and vinblastine. The synthesis of some related anal and derivatives and the preparation of radiolabeled vincristine is being undertaken. In addition, the contract provides for the final isolation, purification and identification of metabolites obtained from samples collec by investigators specified by the National Cancer Institute after in vivo administration of the radiolabeled vincristine.

CALIFORNIA, UNIVERSITY OF (NO1-CM0-2092)

This contract was designed to provide HL-A typing analyses on patients and their families as well as typing of selected donors from the Clinical Cente Blood Bank and all platelet donors from the Central Blood Service of Baltim To date a total of 3,193 typings have been performed. All typings were don and entered into the computerized file on the same day that the blood was received. Thus, HL-A typing results for persons bled in Bethesda were consistently available through the computer terminal by the end of the next day. A total of 590 sera was tested against a panel of 100 standard cells for which HL-A specificities were known during the past contract year.

CALIFORNIA, UNIVERSITY OF (NO1-CM4-3791)

The goal of this project is to develop and use assays which are capable of detecting and evaluating antitumor agents which can increase the lethal eff of ionizing radiation in vitro and in vivo. Such effects will be evaluated by cytocidal activity in cell culture, by effects on the cell's ability to repair x-ray induced DNA damage and by effects on the growth of locally implanted Lewis lung carcinoma in vivo.

CALIFORNIA, UNIVERSITY OF (NO1-CM4-3798)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

CALIFORNIA, UNIVERSITY OF (LOS ANGELES) (NO1-CM5-3752)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

CALIFORNIA, UNIVERSITY OF (SAN FRANCISCO) (NO1-CM6-7055) (FORMERLY NO1-CM4-3706)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

CAPETOWN, UNIVERSITY OF (NO1-CM3-3738)

This contract was designed to conduct studies of intensive multidisciplinary therapy of patients with bronchogenic carcinoma and to determine the efficacy of each of a number of therapeutic approaches, possible relationship to morphologic type and clinical stage of the disease, and the influence of concomitant pathology. It was initiated because of important therapeutic leads which might prolong longevity of patients with lung cancer as the most commonly lethal malignant tumor in the United States as well as many other countries.

CATHOLIC MEDICAL CENTER OF BROOKLYN & QUEENS, INC. (NO1-CM2-3703)

This contract was initiated to provide an experimental model for human breast cancer therapy. The assay system utilized is the CD_8F_1 spontaneous and first-generation transplanted tumor. The drugs being tested are those specified by the Division of Cancer Treatment staff and are those with established clinical activity and candidates for clinical trial. The contract is designed to provide a human tumor model which will enable us to: (1) test drugs to determine those with specificity against breast carcinoma, (2) study the

CATHOLIC MEDICAL CENTER OF BROOKLYN & QUEENS, INC. (NO1-CM2-3703) (CONTIN

influence of treatment schedule on "slow-growing" tumors, and (3) study dr combinations as well as drugs combined with surgery and immunotherapy.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-004-1)

The Contractor has a very active synthetic program preparing nitrogen het cyclic compounds including alkaloid analogs and natural product modificati In addition, native Indian medicinal plants are collected, and the extract are prepared and submitted to the National Cancer Institute screening program for antitumor evaluation.

CHARLES RIVER BREEDING LABORATORIES, INC. (NO1-CM5-3812)

This primary genetic center has as its objectives the development of germ- foundation colonies of inbred rodents required for program studies. Pedig animals are derived via hysterotomy and foster-suckled in germ-free isolat Selected pedigreed offspring are artifically contaminated with pure cultur of organisms and are developed as pedigreed expansion colonies in a variet of isolators. Offspring from this second stage are issued to secondary ge centers which, in turn, produce breeding animals for large-scale productio colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. A large-scale production colony is maintained in order to provide suitable numbers of rodents for laboratory investigators. Task 1 is concerned with the production of germ-free rodents, counterparts with defined microbial f and large-scale production; Task 2 in Calco, Italy, produces rodents in bi containment environments; and Tasks 3 and 4 are concerned with the product of rodents for specific investigations.

CHARLES RIVER BREEDING LABORATORIES, INC. (NO1-CM5-3848)

This rodent production center contract supports a production effort design to furnish animals as required by laboratory programs. Breeding animals a furnished by the Government. Major emphasis is directed toward AKR mouse production.

CHARLES RIVER BREEDING LABORATORIES, INC. NIH-75-C-598)

This procurement contract is designed to furnish 312,000 six-week-old CD2F (BALB/c♀ x DBA/2♂) hybrid mice free of pseudomonads for Drug Research and Development Program contract studies. The breeding animals originate from the genetic center at this site.

CHEMICAL ABSTRACTS SERVICE (NO1-CM4-3722)

The Chemical Abstracts Service (CAS) has participated in installing and is operating the new Chemical Information System in support of the Drug Research and Development Program accessioning and screening activities. This provides the improved capability to: (1) identify which accessions are new to the Drug Research and Development Program and which duplicate or are closely related to known active compounds; (2) produce computer-generated shipping documents and other communications (including structural diagrams) to suppliers, screeners, in-house staff, and the acquisition and storage and distribution contractors who are involved in the accessioning and screening process; (3) maintain inventory control; (4) maintain management and operational control of the accessioning process; and (5) search the files on-line to secure an immediate answer for a possible duplicate sample, for analogs of a compound of interest, or for a management inquiry. CAS is evaluating and installing various system enhancements which will increase the effectiveness and efficiency of searching, reporting, and correcting the file. Support is also provided in identifying for acquisition compounds in the literature which display biochemical or other biological characteristics which may correlate with antitumor activity.

CHESTER BEATTY RESEARCH INSTITUTE (NO1-CM4-3736)

The principal objective of this project is focused on studies of (1) the growth of human tumors in immunologically deprived mice, and (2) the utilization of these xenograft systems as well as established transplantable mouse tumors for the detailed evaluation of drugs of clinical interest. Human tumors are grown in either CBA/Lac mice made immunologically incompetent by x-irradiation and thymectomy (T-lymphocyte depletion) or in athymic ("nude") mice. The Division of Cancer Treatment has identified the development of laboratory models for assessing chemotherapeutic effects against human tumor xenografts as a major program objective. Tasks conducted under this contract are designed to define criteria for the successful xenografting of human tumors to animals utilizing patient resources of the Royal Cancer Hospital, London. As an ancillary task, this contract resource is used to acquire potentially effective new anticancer drugs from the British pharmaceutical and chemical companies and from universities; the Contractor's position in this regard is unique because the senior scientific staff serve as consultants to cancer chemotherapy investigators throughout Europe. Compounds so acquired are submitted to the Division of Cancer Treatment for antitumor screening.

CHICAGO, UNIVERSITY OF (NO1-CM3-3748)

This contract is intended to support studies to isolate and compare the dihydrofolate reductase from normal and malignant tissues, primarily solid tumors, in order to determine any detectable molecular differences in these enzymes. The study also utilizes in vitro testing to help design inhibitors of human and subhuman dihydrofolate reductase. These are aimed at understanding the mechanism of resistance developed in human malignancy to antifols.

CHILDREN'S HOSPITAL OF LOS ANGELES (NO1-CM4-3803)

This contract was designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Two drugs, diglycoaldehyde, NSC-118994, and dianhydrogalactitol, NSC-132313, have been studied in children with acute leukemia and solid tumors.

CHILDREN'S HOSPITAL OF LOS ANGELES (NO1-CM5-3831)

This contract is designed to investigate the role of protected environments and prophylactic antibiotics as adjuvants to the remission induction chemotherapy of stages III and IV neuroblastoma, rhabdomyosarcoma and other small-cell sarcomas. Trials were recently begun but early results are not yet evaluable.

CLINICA NEUROCHIRURGICA DELL UNIVERSITA (DI PAVIA, ITALY) (NO1-CM6-7056)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

COLLABORATIVE RESEARCH, INC. (NO1-CM1-2326)

The objective of this contract is the design and synthesis of oligo- and polynucleotides as potential inhibitors of RNA-dependent DNA polymerase and other DNA polymerases. It is anticipated that the synthetic nucleotides may act as templates and primers for different enzymes and may inhibit viral and leukemic DNA polymerases specifically without interfering with the normal nucleic acid metabolism of the host cells. This will be the terminal year for this contract.

COLLABORATIVE RESEARCH, INC. (NO1-CM6-0506)

The major objective of this contract is the synthesis of some potential metabolites of cyclophosphamide and phosphoramide mustards as latent cytotoxic agents. In addition, the preparation of some analogs and derivatives of cyclophosphamide is being undertaken.

COLLEGE OF MEDICINE AND DENTISTRY OF NEW JERSEY (NO1-CM4-3760)

This contract has as its major purpose serological diagnosis for the presence or absence of infectious mouse ectromelia, LCM, polyoma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, SV5, Toolan's H-1, and the Rauscher and Moloney viruses. The Contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; antibody-producing capacity of tumor-bearing mice and the effect of the route of injection on antibody levels; and a comparison of the sensitivity of caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "passenger" agents on the growth of tumors used in the screen and on their effects on drug action. During a contract period of one year, this laboratory performs approximately 65,000 rodent serum and 6,000 tumor fragment diagnostic tests. The Contractor also prepares large quantities of vaccinia virus used for immunizing mice against infectious ectromelia. This contract will terminate May 1976.

COLORADO STATE UNIVERSITY (NO1-CM1-2192)

The objective of this research-type contract is to investigate the pathogenesis of adriamycin/daunomycin-induced cardiotoxicity utilizing clinical, morphological and biochemical parameters. The rabbit appears to be the most promising species for assessing the cardiotoxicity of this class of anticancer agents. Studies have been designed to establish optimal dose and time schedules to provide the highest incidence and maximum consistency of cardiotoxicity. Adriamycin analogs are being screened for cardiotoxicity using the rabbit with the hope of finding one with a maximum therapeutic effect and minimal cardiotoxicity. The rabbit is also being used for antidotal studies.

COLORADO, UNIVERSITY OF (NO1-CM1-2186)

The purpose of this research and development contract is two fold: (1) to study tRNA and mRNA methylases of normal and tumor tissue, to isolate and purify them to test natural and synthetic inhibitors of the enzymes from the two sources, and (2) to develop biochemical markers which stem from the turnover of tRNA and which are present in elevated levels in the blood and urine of patients with a variety of cancers. Accurate quantitative methods of measurement of four different markers have been developed including dimethylguanosine, methylinosine, pseudouridine and the unusually interesting marker, β-aminoisobutyric acid which can serve as a probe for the metabolism of DNA and transfer RNA from which it originates.

COLORADO, UNIVERSITY OF (NO1-CM4-3779)

This contract is for the chemical synthesis of a number of new and novel nitrogen heterocyclic compounds as potential anticancer agents. The major emphasis is on preparing compounds containing the triazepine and oxathiazine

COLORADO, UNIVERSITY OF (NO1-CM4-3779) (CONTINUED)

ring systems. All materials prepared, including intermediates, are submitt in adequate quantities for antitumor testing. This contract will terminate during this contract period.

COMMUNITY BLOOD AND PLASMA SERVICE (NO1-CM4-2150)

The purpose of this contract is to provide quadruplet units of HL-A typed platelets primarily to the leukemia service of the National Cancer Institut The use of HL-A matched platelets decreases the risk of sensitization or production of antibodies to white cells and platelets, therefore, providin efficient and effective platelet support therapy.

CONNECTICUT, UNIVERSITY OF (NO1-CM1-2181)

The purpose of this contract is to provide clinical data from selected pati with diagnosed brain tumors in order to evaluate the efficacy of therapy. single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisit of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experiment and control groups.

CONTROL DATA CORPORATION (NO1-CM3-3739)

The purpose of this contract is to provide data processing and computer sup which enables simple, rapid and on-demand retrieval of clinical information related to the patient data base provided by the participating group of contractors, the Brain Tumor Study Group, who enter patients on study accor to specified protocols.

CORDOVA CHEMICAL COMPANY (NO1-CM5-7031)

This service preparative contract provides for the resynthesis of a variety compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is dev to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

DOT SYSTEMS, INC. (NO1-CM5-3774)

This service contract was established to provide the necessary administrati and technical support to the Office of the Director, Division of Cancer Treatment. The primary function is the coordination and technical skills involved with the organization of conferences, graphic support and editoria services.

DOW CHEMICAL COMPANY (NO1-CM2-3712)

This service-type contract is concerned primarily with the reproductive studies of three antineoplastic agents in accordance with the FDA Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use, dated January 1966. These studies are divided into three phases: (1) Phase I - general reproductive performance and fertility utilizing mice or rats of both sexes; (2) Phase II - teratology studies using mice and/or rats and rabbits; and (3) Phase III - perinatal studies employing mice and/or rats.

DOW CHEMICAL COMPANY (NO1-CM3-3724)

Some derivatives of rifamycin have been reported to have significant inhibitory activity for viral and cellular DNA polymerases (including reverse transcriptase). The work of this contract is designed to synthesize rifamycin derivatives which are more effective inhibitors of RNA-dependent DNA polymerases. Additionally, the development of a strong selective inhibitor of RNA-dependent DNA polymerases could help in clarifying the mechanism of action of this enzyme and possibly lead to new cancer chemotherapy agents through chemical modifications of 3-formylrifamycin-SV. This contract will terminate during this contract period.

DOW CHEMICAL COMPANY (NO1-CM5-3768)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

DUKE UNIVERSITY (NO1-CM2-2044)

The major objectives of this contract were (1) the development of measures of sensitivity and quantity in autoradiography, and (2) the improvement of sensitivity and resolution of the autoradiographic process. Because of loss of key personnel at Duke University, completion of data acquisition phase of this contract was delayed. Accordingly, the contract was extended without additional funds in order to permit Duke personnel to complete this phase, employing the autoradiographic grain counter developed at the National Cancer Institute. This contract was terminated December 1975.

DUKE UNIVERSITY (NO1-CM6-7010) (FORMERLY NO1-CM0-2030)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in

DUKE UNIVERSITY (NO1-CM6-7010) (FORMERLY NO1-CMO-2030) (CONTINUED)

accordance with the protocols of the Brain Tumor Study Group. The acquisi of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimen and control groups.

EMORY UNIVERSITY (NO1-CM4-3764)

This contract provided support to perform Phase II studies of the response rate of hepatocellular carcinoma, cholangiocellular carcinoma, gallbladder carcinoma and metastatic bioduct carcinoma to adriamycin and other drugs; correlated response of alpha-feto protein and human chorionic gonadotropin titers with clinical antitumor response; and compared response rate of the diseases in Zambian Bantu patients with that of United States patients. T contract terminated December 1975.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION, (YO1-CM1-0056) (FORMERLY ATOMIC ENERGY COMMISSION)

The principal objective of this project is to study the cell cycle phase specificity of clinical drugs and drugs in development toward clinical tri Drugs are evaluated for their ability to selectively inhibit progress thro G_1, S, G_2, and M- phases of their proliferative cycle and for their select cytocidal effects in these phases, as well. Both synchronized and exponen growing mammalian cell culture systems are used. The importance of such s stems from the need to identify and develop drugs which may be specificall cytotoxic to non-proliferating but viable and clonogenic cells. The in vi methodologies used were developed by the Contractor.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (YO1-CM1-0060) (FORMERLY ATOMIC ENERGY COMMISSION)

This contract involves the study of potential biologic markers for cancer body fluids by means of high resolution, high-pressure ion exchange chroma tography. Initial efforts have focused on minor and methylated nucleic ac derivatives (tRNA catabolic end products) in urine. Sensitive analytical tools utilized in addition to high-pressure chromatography include gas chromatography, gas chromatography-mass spectrometry, and high- and low- resolution mass spectrometry. Preliminary quantitative results indicated elevated levels for specific nucleosides in the urine of approximately 60% of the cancer patients studied. Further efforts are focused on the analys of specific serum protein bound sugars (utilizing high-pressure liquid chromatographic separation) in the blood of patients with malignancies. I addition, studies are in progress to determine the compounds represented b abnormal peaks obtained on chromatograms from patients with various select types of cancer.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (YO1-CM4-0101)
(FORMERLY ATOMIC ENERGY COMMISSION)

The objective of this effort is to develop a blood cell separation method which will collect sufficient granulocytes (~10^{10}/day) from normal donors to permit widespread adoption of this technique in granulocyte replacement therapy. A unique design approach which was the end result of an extensive ORNL analysis "Blood Cell Separator Development for In Vivo Leukapheresis" is being followed. This consists of radically modifying the blood flow path of the existing IBM rotor to mechanically enhance rouleax formation and to photoelectrically monitor and control separating cell fractions in the interior of the rotor. This feasibility effort covers rotor fabrication and testing with dog models.

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (YO1-CM4-0102)
(FORMERLY ATOMIC ENERGY COMMISSION)

This agreement will accomplish research and development with instrumental and biological techniques to progressively adapt a prototype cell microfluorometer and computer data analysis system for human tumor cell characterization and drug evaluation studies planned by the National Cancer Institute. The initial year will accomplish the engineering, fabrication, testing and delivery of a multi-parameter cell microfluorometer and sorting device coupled with a PDP-11/40 computer system as well as interim interface device for data collection and analysis. Also to be conducted in parallel are biological studies concerning "instrument compatible" cell staining, dispersal and fixation techniques on untested cell samples from a variety of human and animal tumors of current National Cancer Institute interest. During the second year, it is planned to continue the cell preparation work to explore and optimize techniques, and to exploit the instrument's potential for characterizing model animal tumors (L1210 ascites, B16 melanoma, Lewis lung carcinoma) as well as several human tumors both in vivo and in vitro. The third year's work will investigate improved sizing techniques for small cells (7-10 microns). This will include feasibility studies of potential sensing approaches and possibly alternate cell fixation techniques for Coulter sensing. Biological investigations will aim at FMF characterization of tumor growth as a function of tumor age or size.

FARMITALIA (NO1-CM5-7014)

The major objective of this contract is the synthesis of analogs of adriamycin and daunomycin modified in the side chain of the aglycone and in the sugar moiety. Modifications on the side chain of the aglycone should give active compounds with different pharmacological properties because an intercalation model shows the atoms of these side chains to be outside the DNA helix and, therefore, not to interfere with intercalation. The purpose of the modifications to the sugar moiety is to reduce the enzymatic hydrolysis of the glycoside lingage and produce compounds with decreased cardiotoxicity.

FARMITALIA (NO1-CM5-7036)

The Contractor's objectives are to develop a practical synthesis of the aglycone of the antibiotic adriamycin. In addition, they will develop synthesis of new related aglycones which will be coupled to daunosamine and other amino sugars. These materials will provide opportunity for structure-activity relationship studies. Such materials will also be used to study the binding characteristics with DNA as the aglycone is the portic of the adriamycin responsible for intercalation. The end objective would be to develop adriamycin-like compounds with significant activity and less toxicity. Information may also be obtained concerning carcinogenic contributing parts of the molecule.

FLOW LABORATORIES, INC. (NIH-75-C-571)

This contract furnishes approximately 312,000 $CD2F_1$ (BALB/c♀ x DBA/2♂) hybr mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

FRANKLIN INSTITUTE (NO1-CM5-3771)

This contract provides for the preparation, printing and distribution of Cancer Therapy Abstracts, Volumes 16 and 17 for 1975 and 1976. Computer tapes of the abstracts and indexes are also supplied. The price of the annual subscriptions pays the cost of printing and mailing by the Contracto This current awareness journal, published monthly, supplies abstracts and annotations of the world's literature pertinent to the treatment of cancer.

GEORGE WASHINGTON UNIVERSITY (NO1-CM1-2206)

Investigations of physiological and biochemical variables in spontaneous lymphosarcoma and certain normal tissues in dogs are being conducted. The effects of methotrexate alone and in combination with calcium leucovorin or these variables are being studied.

GEORGETOWN UNIVERSITY (NO1-CM1-2123)

The objective of this program is to conduct virologic studies of patients participating in the National Cancer Institute's chemotherapeutic program to make possible evaluations that will aid the clinician in the selection of measures for supportive care and allow for the development of informatio on the importance of the role of viruses in the cancer patient in both protected and conventional environments.

GEORGETOWN UNIVERSITY (NO1-CM3-3745)

The Eastern Cooperative Oncology Group consists of 350 members and 35 cooperative institutions plus subsidiary hospitals. The Operations Office

GEORGETOWN UNIVERSITY (NO1-CM3-3745) (CONTINUED)

funded by this contract, provides executive and administrative support for activities of the Group. On request from investigators, provides randomization and Group correspondence; obtains data from the investigators and maintains liaison with appropriate members of the National Cancer Institute staff. The Contractor has continued to function well during the past contract year and has been responsible for changing the activities of the ECOG along more disease-oriented lines.

GEORGETOWN UNIVERSITY (NO1-CM4-3732)

This contract has as its main objective the production of novel actinomycins by using biotransformation and co-metabolism fermentation techniques. These novel actinomycins will be produced in 100-500 mg. quantities and made available for broad-tumor screening. The use of many different compounds as substrates for the actinomycin fermentations will be thoroughly explored.

HARLAN INDUSTRIES, INC. (NO1-CM2-3218)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. All activities are performed in bio-containment environments. This contract terminates June 1976.

HARLAN INDUSTRIES, INC. (NIH-75-C-591)

This contract furnishes approximately 312,000 six-week-old $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

HARVARD UNIVERSITY (NO1-CM5-3825)

This project is based upon the exploration of the possibility that disarming malignant cells by increasing their immunogenicity by altering their cell surface polysaccharide composition, may be an effective chemotherapeutic approach. A detailed study of the effects of naturally occurring sugars and of sugar analogs on the oncogenicity of tumor cells and on the composition of their membrane glycoproteins, is being performed.

HAWAII, UNIVERSITY OF (NO1-CM3-3747)

This contract provides for the collection of plant samples from among the indigenous and exotic higher plants of the Hawaiian Islands and the recollection of larger samples of those that are reproducibly active in the Drug Research

HAWAII, UNIVERSITY OF (NO1-CM3-3747) (CONTINUED)

and Development Program tests. Plants so obtained are shipped to the extraction contractor, WARF Institute, Inc., and the extracts tested at one of our contract screeners. Larger recollections are shipped to the individual fractionators as assigned by the Drug Research and Development Program.

HAZLETON LABORATORIES, INC. (NO1-CM2-3701)

Mixed leukocyte cultures are an important determinant of the degree of compatibility of two individuals relative to transplantation antigens. The main objective is the continued immune evaluation of patients on chemotherapy using this technique. In addition, a large liquid nitrogen freezer for storage of blood and bone marrow samples is available.

HAZLETON LABORATORIES, INC. (NO1-CM3-3705)

This contract provides for microbiological services in support of the National Cancer Institute, Division of Cancer Treatment, clinical investigations at the Baltimore Cancer Research Center, and for special testing pertinent to this program. The services have also included microbiological surveillance and environmental studies in support of the Laminar Air Flow Room protocols of the National Cancer Institute, National Institutes of Health, Bethesda, Maryland. Preliminary results of the studies are teletyped to the BCRC and NCI daily and used as indicated in research microbiological management of patients. The program employs a dataphone system for presumptive reporting and computer techniques for the retrieval of information as required by clinicians and research investigators, as well as final summarized microbiological reports for patients' medical records.

HAZLETON LABORATORIES, INC. (NO1-CM3-3708)

The major objectives of this contract are: (1) to obtain comparative data on the response of primates to known rodent carcinogens and to materials suspected of being carcinogenic in man; (2) to develop biological markers and diagnostic tests for detecting pre-neoplastic changes as well as frank neoplasia; (3) to obtain model tumor systems in primates; (4) to evaluate the long-term effects of antineoplastic agents which are being used clinically for long-term remissions and in the treatment of diffuse collagen disorders; (5) to make available normal and tumor-bearing animals for pharmacologic, toxicologic, biochemical and immunological studies; and (6) to maintain a breeding colony of various species of primates so that these primates may be readily available for use. All work is carried out in close cooperation with the Laboratory of Chemical Pharmacology, National Cancer Institute.

HAZLETON LABORATORIES, INC. (NO1-CM4-3702)

This contract provides for the supply of dogs for leukocyte transfusion and transplantation experiments. The Contractor supplies animals with spontaneous neoplasms in order to evaluate the immunotherapeutic effectiveness of bone marrow transplantation and immunotherapeutic manipulation following chemotherapy regimens.

HAZLETON LABORATORIES, INC. (NO1-CM4-3705)

The contract performs studies to determine the pharmacologic and toxicologic parameters of antineoplastic agents which are administered intrathecally. Appropriate drugs of either routine preparation or special formulation are provided by the National Cancer Institute, Drug Research and Development Program. The exact treatment schedules and experimental details are determined by the Project Officer in coordination with the Principal Investigator. Every effort is made to keep protocols consistent so that comparison between drugs may be made.

HAZLETON LABORATORIES, INC. (NO1-CM5-7007)

New materials, both synthetic and of natural product origin, as well as fractions of crude natural products are tested in vivo at this contract laboratory. The current testing level is at a rate of 25,000 tests per year. Both crude natural products and new synthetic materials are pre-screened in the P388 lymphocytic leukemia. Confirmed presumptive actives as well as treatment schedules may be tested in the L1210 leukemia, B16 melanoma, Lewis lung carcinoma, or the intracerebrally implanted ependymoblastoma at the direction of the Drug Evaluation Branch staff. The revised Drug Research and Development Program protocols are followed for all testing. Research and development work is conducted as requested by the Drug Evaluation Branch.

HAZLETON RESEARCH ANIMALS, INC. (NIH-76-C-0125)

This contract provides 300 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

HEBREW UNIVERSITY (HADASSAH MEDICAL SCHOOL) (NO1-CM1-2127)

This research contract is concerned with the effects of an extract, MER, (methanol extractable residue), obtained from attenuated tubercle bacilli (BCG) in stimulating the immune mechanism in animals and man with particular emphasis on antitumor activity.

IIT RESEARCH INSTITUTE (NO1-CM4-3755)

This represents a support services contract to provide assistance to the Drug Evaluation Branch staff in preliminary data evaluation and follow-up on materials found active in the presumptive screen. The Contractor participates with staff in monitoring testing and in expediting work on those materials which appear to have the greatest promise, based upon initial activity.

IIT RESEARCH INSTITUTE (NO1-CM5-3829)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of Salmonella spp. and Pseudomonas spp. in program rodents and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. The scope of performance of this contract was changed recently to the extent that they are now monitoring animals leaving the genetic and rodent production centers primarily for the presence of ecto- and endoparasites. They also forward blood serum samples to the College of Medicine and Dentistry of New Jersey where these same animals are monitored for virus diseases. The results of these monitoring studies will be utilized to upgrade the quality of animals being sent to the laboratories. Diagnostic procedures are also carried out at this facility when disease problems occur in the various animal breeding colonies or in the laboratories using these animals.

IIT RESEARCH INSTITUTE (NO1-CM5-7004)

This contract provides for the performance of in vivo testing of new synthetic materials and natural products. Screening is being conducted according to Drug Research and Development Program protocols at a level of approximately 50,000 tests per year. New natural products and synthetics are being tested in the P388 leukemia in the mouse host. Testing is conducted in the B16 melanoma, Lewis lung carcinoma and ependymoblastoma tumor systems when requested by Drug Research and Development Program staff. Work is continuing with a murine renal carcinoma as a potential organ-targeted system, and methodology has started on murine colon tumors for secondary screening. All methodology and development is carried out only at the request of the Project Officer and during this year has been devoted to the development of new in vivo screening tumor test systems.

IIT RESEARCH INSTITUTE (NO1-CM6-3832)

This new contract for cell culture screening of new substances for cytotoxicity was awarded December 1, 1975 as the result of competition. Standardized cell culture assays are performed in accordance with procedures described by the Drug Research and Development Program protocols as an aid in the isolation and purification of potential antitumor agents from natural plant or animal sources. Selected synthetic materials are also screened as required by the Program. In consultation with the Project Officer, the Contractor also participates in the development and standardization of new cell culture test systems.

ILLINOIS, UNIVERSITY OF (NO1-CM2-2078)

This contract provides for the fractionation of confirmed active plant extracts and fermentation products in an attempt to isolate in a pure state and identify the active compound(s). Plant materials used in this work are obtained, for the most part, through the U. S. Department of Agriculture.

ILLINOIS, UNIVERSITY OF (NO1-CM2-3208)

The principal objective of this contract is to provide the samples (not previously available) of a number of ansamycin-type antibiotics for biological and related testing. This group includes the streptovaricins, tolypomycins and geldanamycins, some of which are highly potent RNA-dependent DNA polymerase inhibitors and also have antiviral activity. This contract is being terminated during this contract period.

INDIAN CANCER RESEARCH CENTRE (PL-480 - AGREEMENT NO. NIH-01-010-1)

This PL-480 Agreement is for the collection and identification of natural products indigenous to India, the preparation of crude extracts from these materials, and the testing in vivo of these extracts as well as synthetic materials synthesized in India.

For this purpose, an in vivo screening laboratory is maintained in Bombay, India together with an animal breeding colony to supply the necessary host animals. Currently, the L1210 lymphocytic leukemia and the P388 lymphoid leukemia are utilized with testing conducted as per current Drug Research and Development Program protocols. The L1210 tumor system is employed for the testing of synthetic materials with the P388 tumor system employed for the testing of natural products. Those active in this system are tested in the L1210 tumor system.

INDIANA UNIVERSITY FOUNDATION (NO1-CM6-7057) (FORMERLY NO1-CM0-2028)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

INSTITUT JULES BORDET (NO1-CM5-3840)

The objective of this contract is the maintenance of a liaison office at the Institut Jules Bordet, Cancer Center of the University of Brussels, Belgium. The program is designed to foster close collaboration between European and United States investigators in the development and application of new

INSTITUT JULES BORDET (NO1-CM5-3840) (CONTINUED)

clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy. In addition to the information activities, an animal tumor test is maintained for the more detailed evaluation and development of selected new compounds, in accordance with National Cancer Institute interests.

INSTITUT JULES BORDET (NO1-CM5-7040)

This is a service contract for the in vivo screening of new synthetic and natural product materials and the follow-up testing of active materials. The primary screening is carried out in accordance with Drug Research and Development Program protocols which require new materials to be tested against the P388 leukemia test system. The current level of testing is approximately 25,000 L1210 equivalent tests per year. As requested, detailed testing is performed with other tumor test systems, such as the L1210 leukemia, the Lewis lung carcinoma and the B16 melanoma, and schedule dependency studies conducted with materials of potential clinical interest. Methodology studies, such as the characterization and evaluation of tumor test systems, are carried out as directed by the Project Officer.

INSTITUTE OF CANCER RESEARCH, ROYAL CANCER HOSPITAL (NO1-CM2-3717)

The objectives of this contract are to study the effects of chemotherapeutic agents in experimental solid tumor systems and in human xenografts transplanted into immune-suppressed mice in relation to their proliferative state and their vascular supply, and compare the effects of these agents on the hematopoietic system. In the past, quantitative in vivo and in vitro clonogenic assays were developed for B16 melanoma and Lewis lung carcinoma and for normal marrow. More recently, in vivo diffusion chamber assays have been developed which will permit comparisons of clonogenic cell populations in mouse and human normal and tumor tissues in the same experimental system. Interrelationships among the effects of nitrosoureas, alkylating agents and radiation have been studied. Studies are in progress comparing cell survival in tumor and marrow following other drugs in single and multiple doses and in two-drug combinations. Radiotracer methods have been developed to measure tumor blood supply. These methods will be used to study proliferative and drug response characteristics in tumors in relation to their blood supply.

IOWA, UNIVERSITY OF (NO1-CM4-3743)

The objectives of this contract are to develop, manufacture and package parenteral dosage forms of investigational drugs for clinical evaluation. This Contractor also has the capability to produce products for oral use, if required. The Contractor is responsible for completing required analytical and safety tests on each product prepared. All products produced are packaged, labeled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI" (NO1-CM2-3242)

Studies with adriamycin and some of its derivatives on their pharmacokinetic behavior in mice bearing solid tumors are being investigated. Changes in pharmacokinetic parameters following multiple dose treatment are being explored. Comparison of in vitro and in vivo drug effects of the various derivatives has begun.

ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI" (NO1-CM5-3826)

This contract is for the purpose of development of immunochemotherapy studies on established or new experimental model systems. The studies include the development of new tumor-host systems in inbred as well as hybrid mice in various genetic combinations as well as studies of combined modalities of antineoplastic immunotherapy and chemotherapy.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (NO1-CM3-3714)

The purpose of this contract is the evaluation of new anticancer drugs alone or in combination with conventional agents in prospective controlled clinical trials. Four studies are being performed: (1) adjuvant combination chemotherapy for breast cancer with positive axillary nodes; (2) controlled study with multiple drug combinations for metastatic breast cancer; (3) controlled study of new chemotherapeutic regimens in advanced carcinoma of stomach, colon and rectum; and (4) a controlled study of BCNU, CCNU and irradiation in the treatment of malignant glioma.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (NO1-CM4-3726)

This contract represents utilization of a unique resource of both advanced and early patients with malignant melanoma for various controlled clinical trials. Dr. Veronesi, the Project Director, is the Chairman of the International Group for the Clinical Study of Melanoma. There are 27 collaborating institutions including Eastern and Western Europe, Australia (Sydney), and South America (Montevideo). Investigators have surgical, pathologic, chemotherapeutic and immunotherapeutic expertise. They have accrued satisfactorily in a protocol comparing prolonged chemotherapy (DTIC), immunotherapy and chemotherapy (BCG + DTIC) as adjuvants to surgery to a control of surgery alone. His tree-arm study was activated June 15, 1974 and in 18 months accrued 111 properly stratified, evaluable patients (head and neck versus upper and lower limb, Npos only; and trunk Npos and N negative and prior treatment). So far, recurrences are not significantly different in all arms. A protocol has been proposed in advanced melanoma designed to evaluate the effect of BCG or C. parvum or DTIC treatment. This is a timely study in view of uncontrolled reports of superiority of chemo/immunotherapy to chemotherapy alone.

JACKSON LABORATORY (NIH-263-76-C-0176)

This service contract provides for the procurement of approximately 78,000 AKR inbred mice to screening contractors for tumor transplantation and as breeders for large-scale hybrid production colonies. Breeding animals are supplied by the Contractor.

JAPANESE FOUNDATION FOR CANCER RESEARCH (NO1-CM2-2054)

The objective of this contract is the maintenance of a liaison office at the Japanese Foundation for Cancer Research in Tokyo. The program is designed to foster close collaboration between Japanese and United States investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer chemotherapy. A small testing facility is also maintained for the screening and further evaluation of selected new compounds.

JOHNS HOPKINS UNIVERSITY (NO1-CM4-3718)

The synthetic and catabolic rates of specific myeloma paraprotein will be studied utilizing radioactive guanidoarginine for labeling purposes in mice bearing a transplanted myeloma cell line. Efforts will be made to correlate total cell number with the above parameters in an attempt to work out a possible method for determining this number. Comparison will be made to an existing system which requires culture of the involved cells to determine synthetic rate.

KANSAS, UNIVERSITY OF (NO1-CM1-2184)

The principal objective of this contract is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease and to analyze these data for individual variability which can be correlated with clinical response or some other pharmacologic parameter. Methotrexate and 5-fluorouracil are currently under study.

KANSAS, UNIVERSITY OF (NO1-CM2-3217)

This contract provides research capabilities in the area of formulation design and development. They receive formulation problems that cannot be resolved by the usual solubilization and/or stabilization methods.

KANSAS, UNIVERSITY OF (NO1-CM4-3802)

This contract was designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute, or to conduct Phase I clinical studies with new combinations or

KANSAS, UNIVERSITY OF (NO1-CM4-3802) (CONTINUED)

regimens mutually agreed upon. Protocols on Phase I studies with dianhydrogalactitol, NSC-132313; anguidine, NSC-141537; and parenteral MeCCNU, NSC-95441, have been conducted.

KANSAS, UNIVERSITY OF (NO1-CM5-3819)

This Contractor maintains a genetic production center. Its most important function is to secure the maintenance of the strains of rodents that are of interest in Drug Research and Development Program investigations. Its spectrum includes three strains: C57BL/6, DBA/2 and BSVR/Sr. Animals not required for perpetuation of pedigree are supplied to screening contractors and as breeders for production colonies. Hybrids for special studies are produced as required for specific studies.

KENTUCKY, UNIVERSITY OF (NO1-CM6-7058) (FORMERLY NO1-CM7-1173)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a useful analysis of comparative clinical course and survival for experimental and control groups.

LABORATORY RESEARCH ENTERPRISES, INC. (NIH-263-76-C-0124)

This contract provides 300 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

LABORATORY SUPPLY COMPANY, INC. (NO1-CM5-3775)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. All activities are performed in bio-containment environments.

LABORATORY SUPPLY COMPANY, INC. (NIH-75-C-577)

This contract furnishes approximately 312,000 CD2F_1 (BALB/c♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

LEEDS, UNIVERSITY OF (NO1-CM4-3727)

The purpose of this contract is the investigation of the usefulness of various "markers" in the study of gastrointestinal cancers with special reference to the earlier detection of metastases and their pattern of change during the evolution of the disease.

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (NO1-CM5-3814)

This primary genetic center has as its objectives the development of germ-free foundation colonies of inbred rodents. Pedigree animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of non-pathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to secondary genetic centers, which, in turn, produce breeding animals for large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

LITTON BIONETICS, INC. (NO1-CM1-2340)

The objectives of this project are (1) to purify viral antigens from RNA tumor viruses and human leukemic cells and to prepare antisera against these antigens; (2) to analyze the nucleic acid relatedness of DNA and RNA from human leukemic cells or of RNA from type-C viruses isolated from human cells to nucleic acids from known primate type-C viruses; (3) to study the effect of various elements, including growth factors and type-C viruses, on the growth and differentiation of hematopoietic stem cells, normal and leukemic; (4) to determine if viral-related macromolecules or other possible leukemia-specific macromolecules will be of value as diagnostic and prognostic tools for human leukemia; and (5) to expand basic research efforts in tumor cell biochemistry in general in order to achieve a better understanding of the neoplastic process and its prevention and treatment.

LITTON BIONETICS, INC. (NO1-CM2-2062)

The main objective of this contract is the preparation, growth and characterization of defined tissue culture cell lines and of fresh human leukemic blood cells for use by Project Officers. Cell cloning, cell genetics, cytochemistry, defining immunological cell markers and labeling cells with radioisotopic precursors for the isolation of metabolic products are also performed.

LITTON BIONETICS, INC. (NO1-CM2-5423)

The Government-owned, contractor-operated Frederick Cancer Research Center serves as a muti-faceted contract involving the following activities for the Division of Cancer Treatment:

LITTON BIONETICS, INC. (NO1-CM2-5423) (CONTINUED)

Project 17

At the Frederick Cancer Research Center, the Division of Cancer Treatment is developing a fermentation pilot plant for the large-scale production and isolation of microbial products of interest to the program. Facilities will also be available for the production of other natural products. A fermentation research laboratory has been completed and will be used to systematically evaluate various microbes' abilities to ferment unique substrates and produce novel compounds.

Project 19

The Frederick Cancer Research Center was selected to establish, operate and maintain a Biologic Markers Program. The individual elements of the program include an analytical services unit and specimen repository with computerized data storage/analysis capability and a basic research unit for markers. The program provides rapid routine analyses on established markers and conducts direct research to find, develop and thoroughly explore the potentials of new markers for specific diseases, e.g., breast, gastrointestinal, lung, ovarian, brain, pediatric, etc., malignancies. Improvement of existing specific tumor markers will also be explored.

LITTON BIONETICS, INC. (NO1-CM3-3741)

This contract provides a source for the screening of selected drugs for inhibitory activity for selected mammalian cell and viral nucleotide polymerase enzymes (in vitro) and for toxicity or specific inhibition of oncogenic viral replication and transforming capacity in cell culture systems. Enzymes employed are the Simian sarcoma virus reverse transcriptase, mammalian DNA polymerases I and II, RNA polymerases I and II and Poly-A polymerase. Cell culture effects on DNA, RNA and protein synthesis, cell proliferation and murine sarcoma virus focus formation in 3T3 cells are measured. The screening operation includes a preliminary cell culture and anti-enzymatic screen for all compounds and a secondary cell culture and anti-enzymatic evaluation for compounds demonstrating significant activity in the primary screen. Materials submitted to these screens are selected by the Project Officer.

LITTON BIONETICS, INC. (NO1-CM5-3778)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of Salmonella spp. and Pseudomonas spp. in program rodents and other materials used for animal husbandry practices. Specimans are forwarded by contract laboratories and animal production establishments. Approximately 10,000 samples will be screened per year. The research portion of this contract (a minor component) is directed towards improvement in cultural techniques.

MADRID, UNIVERSITY OF (NO1-CM5-3792)

The object of this contract is to evaluate the effects of antitumor drugs on the electron transport processes in aerobic energy metabolism. The systems used are isolated heart and liver mitochondria, and intact Ehrlich ascites cells, using O_2 electrode measurements of oxygen consumption as well as spectroscopic methods to determine the oxidation state of electron transport chromophores. In addition, the action of selected drugs on myocardial membrane Na-K dependent ATPase is being evaluated.

MAKERERE UNIVERSITY COLLEGE (NO1-CM7-1343)

This contract was developed to operate the Lymphoma Treatment Center and the Solid Tumor Center, collectively known as the Uganda Cancer Institute, to provide facilities for direct collaborative studies to the members of the National Cancer Institute, including the Clinical Oncology area, Laboratory of Pharmacology, Immunology, Pathology and Etiology. Research is continuing under the direction of Dr. Sebastian Kyalwazi and Dr. Charles Olweny.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (NO1-CM3-3720)

The main purpose of this project is to establish a regional European contract as an extension of the Division of Cancer Treatment compound acquisition and screening program. The Contractor collects approximately 1,500 materials in Southern European countries and performs primary screening at a rate of approximately 7,500 L1210-equivalency tests per year.

MARSHALL RESEARCH ANIMALS, INC. (NIH-263-76-C-0123)

This contract provides 300 beagle hounds for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

MARYLAND, UNIVERSITY OF (NO1-CM4-3748)

Under this service contract, the University of Maryland at Baltimore provides suitable facilities, administrative support, ancillary support services and personnel for the inpatient operation of the Baltimore Cancer Research Center, as well as space for activities such as outpatient department, cell component therapy, patient care pharmacy and laboratory efforts necessary for the conduct of the program.

MASON RESEARCH INSTITUTE (NO1-CM5-7003)

This service contract is for the in vivo testing of synthetic and natural products which are submitted to the Drug Research and Development Program. The P388 tumor system is utilized for the testing of synthetic materials.

MASON RESEARCH INSTITUTE (NO1-CM5-7003) (CONTINUED)

Natural products are tested initially in the P388 leukemia with those demonstrating efficacy being tested in the L1210 leukemia. Testing is proceeding at a level of approximately 37,000 L1210 equivalent tests per year. Schedule dependency studies to determine the optimal dose, schedule and route of administration, combination studies and special studies on other tumor test systems are conducted as directed by Drug Research and Development Program staff.

MASON RESEARCH INSTITUTE (NO1-CM5-7030)

This contract is designed to evaluate and develop new experimental solid tumor models with potential predictive value in selecting drugs which will be active against human solid tumors. Emphasis will be placed on human tumor xenografts. A colony of athymic nude mice will be established and a number of human tumor cell lines will be established and maintained by serial transplantation. The Contractor will evaluate biological characteristics such as growth, cytokinetics and metastasis and determine the response to established and potential antitumor agents.

MASON RESEARCH INSTITUTE (NO1-CM6-7011)

This contract supports the Clinical Oncology Program of the Division of Cancer Treatment with computer programming expertise for the development of clinical information systems and with data technician services for the maintenance and utilization of these systems. A wide variety of systems have been developed and are maintained for the clinical Branches of the Division of Cancer Treatment.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CM4-3712)

The objectives of this project are (1) the unique conceptual division of the bleomycin molecule into five complex but synthetically obtainable intermediates, (2) the synthesis of each of the unique parts for biological testing and comparison with the analogous part from the natural product where possible, and (3) the joining of the five parts, one at a time in the proper sequence, in order to determine whether all five parts are necessary for antitumor activity. The Contractor will submit samples of the intermediates as well as final products for in vivo and in vitro evaluation by the National Cancer Institute. These compounds will be fully characterized as to purity and structure.

MAYO FOUNDATION (NO1-CM0-2066)

The purpose of this contract is the investigation of new drugs in the treatment of advanced gastrointestinal tumors as a cooperative venture between the Mayo Foundation and the National Cancer Institute. The objective is to have

MAYO FOUNDATION (NO1-CMO-2066) (CONTINUED)

each drug evaluated in a minimum of 20 cases of adenocarcinoma of the colon to meet the requirements of the Division of Cancer Treatment program linear array. Provision has been made for reporting of data on each trial in a manner which is consistent with the data retrieval currently employed intramurally at the National Cancer Institute. This will enable both direct comparisons and possible integration of data on new drugs from both of these sources.

MAYO FOUNDATION (NO1-CM4-3715)

This contract is designed to undertake combined modality clinical studies in testicular tumors. The initial protocol treats Stage II nonseminomatous lesions with surgery followed by random allocation to radiotherapy, chemotherapy or radiotherapy plus chemotherapy.

MAYO FOUNDATION (NO1-CM4-3783)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

MAYO FOUNDATION (NO1-CM4-3796)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

MAYO FOUNDATION (NO1-CM5-3772)

This contract is designed to support prospective randomized, controlled studies using combination chemotherapy in the treatment of breast cancer.

MAYO FOUNDATION (NO1-CM5-3838)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Dianhydrogalactitol, NSC-132313, has been studied in adult patients with solid tumors. Protocols for Phase I studies with butocin, NSC-172755, and hycanthone, NSC-134434, have been accepted.

MAYO FOUNDATION (NO1-CM5-7033)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MAYO FOUNDATION (NO1-CM5-7044)

This contract is designed to support Phase II-III studies in solid tumors, by means of prospective randomized, controlled clinical trials involving the use of combined modalities.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM4-3716)

This contract was designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Piperazinedione, NSC-135758, has been studied in solid tumors in adults and children.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM4-3804)

This contract has been designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies with piperazinedione, NSC-135758, and thiadiazole, NSC-4728, have been conducted.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM5-3844)

This contract is designed to support Phase II-III studies in solid tumors by means of prospective randomized, controlled clinical trials involving the use of combined modalities.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM5-7019)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM5-7043)

This contract is designed to support Phase II-III studies in solid tumors, by means of prospective randomized, controlled clinical trials involving the use of combined modalities.

MIAMI, UNIVERSITY OF (NO1-CM4-3800)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

MIAMI, UNIVERSITY OF (NO1-CM5-7041)

The objectives of this contract are to measure the immunosuppressive effects of drugs in order to determine how time and dose variables of treatment influence the depth and duration of suppression. These studies employ existing in vitro systems to determine the effect of the various anti-neoplastic agents on humoral and cell-mediated immune responses.

MIAMI, UNIVERSITY OF (NO1-CM6-7077) (FORMERLY NO1-CM0-2225)

Synthetic materials of too small a quantity for in vivo testing, crude natural products, and fractions of crude natural products that have shown efficacy in either in vivo or in vitro Drug Research and Development Program tumor systems are tested in vitro at this laboratory. The KB tumor system as well as both L1210 leukemia and P388 lymphoid leukemias are employed. Drug Research and Development Program protocols are followed and research and development is conducted at the request of the Project Officer. In vitro testing is conducted at a 15,000 9KB test equivalent level.

MICHIGAN CANCER FOUNDATION (NO1-CM5-3789)

The objective of this project is to characterize the range of molecular biological effects of antitumor drugs. In addition to effects on nucleic acid synthesis, the effects of new drugs on DNA structure, cell membrane permeability, cell surface electrical charge, cell surface adhesive properties, and enzymes involved in the synthesis of cell surface glycoproteins will be studied.

MICHIGAN DEPARTMENT OF HEALTH (NO1-CM3-3702)

This contract provides assay services utilizing a variety of cell culture methods as aids in the purification of potential antitumor agents from fermentation broths. The methods employed include paper chromatography and paper electrophoresis in conjunction with cell culture agar diffusion

MICHIGAN DEPARTMENT OF HEALTH (NO1-CM3-3702) (CONTINUED)

bioautography, as well as growth inhibition assays, both in stationary and suspension cultures. The related fermentation and isolation program serviced by this assay contract is financed by the Michigan Department of Health.

MICHIGAN, UNIVERSITY OF (NO1-CM9-2004)

This contract is for the chemical synthesis of a number of new nitrogen heterocyclic compounds containing complex ring systems. The compounds are for primary screening and are unique structures which have been little explored and/or are heretofore unknown. All materials are fully characterized, of high purity and in sufficient quantity for adequate evaluation.

MICROBIAL CHEMISTRY RESEARCH FOUNDATION (NO1-CM5-7009)

The major objective of this fermentation contract is to evaluate marine actinomycetes and other cultures for their ability to produce novel agents which inhibit specific biochemical reactions. These agents will be isolated, identified and evaluated in our animal tumor test systems.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM4-3761)

The major objective of this contract is the synthesis of new potential anti-tumor agents. The synthesis is directed at following up active leads from natural and synthetic sources. The areas of investigation include the preparation of analogs and derivatives of natural products and active synthetic compounds. All materials prepared, including intermediates, are submitted for antitumor testing.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM4-3793)

The function of this contract is to provide the National Cancer Institute with services for the storage and distribution of bulk drugs and chemicals to various research laboratories throughout the country. The contract also provides for the weighing, packing and shipping of chemical samples and the maintenance of accurate inventory records.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM5-3818)

This genetic production center supplies inbred rodents for tumor transplantation, for hybrid production, and for compound evaluation studies. Animals are supplied from a colony of two strains of rodents.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM6-3810)

This contract represents a continuation of a five-year effort to measure histocompatibility of platelet and leukocyte transfusions. The Contractor has performed several leuko-agglutinin assays and lymphocyte cross match studies in order to insure safe and reliable blood component transfusions. These efforts are used in the daily management of the National Cancer Institute blood transfusion program.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM6-7069)

This contract has as its major purpose serological diagnosis for the presence or absence of infectious mouse ectromelia, LCM, polyoma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, SV5, Toolan's H-1, and the Rauscher and Moloney viruses. The Contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; antibody-producing capacity of tumor-bearing mice and the effect of the route of injection on antibody levels; and a comparison of the sensitivity of caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "passenger" agents on the growth of tumors used in the screen and on their effects on drug action. During a contract period of one year, this laboratory performs approximately 65,000 rodent serum and 6,000 tumor fragment diagnostic tests. The Contractor also prepares large quantities of vaccinia virus used for immunizing mice against infectious ectromelia.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CM9-2177)

The Contractor performs routine serologic assays for the determination of alloimmunization on National Cancer Institute patients and potential platelet and leukocyte donors. The Contractor serves as a serum repository for serialized serum specimens from all patients on the Pediatric Oncology Branch and has continued to perform well.

MIDWEST RESEARCH INSTITUTE (NO1-CM3-3722)

Midwest Research Institute is one of the two facilities where analyses of experimental medicinal compounds and their clinical formulations are performed for the Drug Research and Development Program. The Contractor determines the identity and purity of these materials, developing procedures for their analysis when standard methods are not available. The techniques employed include elemental analysis, chromatographic and spectrometric procedures, and other physiochemical procedures appropriate to the situation. This contract provides the necessary analytical data for filing with the Food and Drug Administration.

MIDWEST RESEARCH INSTITUTE (NO1-CM3-3743)

Midwest Research Institute is one of three major synthetic contractors for the Drug Research and Development Program. The primary objective of this contract is the rapid exploitation of leads generated by the Drug Evaluation Branch and clinical groups. This Contractor has provided a large number of compounds to the Drug Research and Development Program. These compounds represent the exploration of a variety of leads. Current investigation centers around the synthesis of (1) potential tRNA O-methyltransferase and protease inhibitors, (2) receptor site-oriented antileukemic compounds based on the N-O-O triangulation hypothesis developed by this Contractor, (3) coralyne, nitidine, bleomycin, streptonigrin, colchicine and other natural products and their analogs, and (4) analogs of procarbazine. The materials prepared have been of high purity, thoroughly characterized and in sufficient quantity for adequate evaluation.

MILES LABORATORIES (NO1-CM4-3709)

This is a no-cost contract concerned with the marketing of a clinically useful drug, DTIC. The Contractor summarized the clinical and other data, prepared and filed the New Drug Application (NDA) with the Food and Drug Administration, and prosecuted the NDA until it was approved. The NDA has been approved and the Contractor has arranged for appropriate marketing and other aspects of assuring continuing supplies of the drug to the public.

MINNESTOA, UNIVERSITY OF (NO1-CM4-3788)

The major emphasis of this project is directed toward determining the cell uptake transport processes of selected antitumor drugs. In addition, the effects of membrane active, natural substances on the uptake and effectiveness of antitumor drugs are being evaluated.

MISSOURI, UNIVERSITY OF (NO1-CM1-2323)

The objective of this contract is to develop procedures involving various advanced high resolution analytical techniques using specific instrumentation (e.g., GLC, amino acid analyzer, mass spectrometer, etc.) to measure quantitatively specific and potential biologic markers in the urine and blood of cancer patients and to utilize these procedures to study patients before, during and after therapy. Methods have been established for assay of polyamines (putrescine, spermidine, cadaverine, and spermine) and methylated nucleic acid derivatives, as well as other minor nucleic acid bases in urine and clinical studies are in progress to determine the value of these markers for following disease status in patients. In addition, preliminary work has been initiated for the assay of several other potential markers in serum and urine including serum fucose/protein ratio, urinary ß-aminoisobutyric acid, and hydroxyproline/creatinine ratio.

MONSANTO RESEARCH CORPORATION (NO1-CM2-3715)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at Monsanto or acquired from other sources, are analyzed for purity and identity by radioautography, assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

MONSANTO RESEARCH CORPORATION (NO1-CM3-3709)

This service preparative contract provides for the large-scale synthesis of compounds required for preclinical and clinical studies. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

MONTEFIORE HOSPITAL (NO1-CM6-7061)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM4-3703)

The objective of this contract is to identify the antitumor activity of new and old agents against advanced Stage II and IV ovarian carcinoma.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM5-3837)

This contract has been designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Protocols for Phase I studies with neocarzinostatin, NSC-157365, and vindesine, NSC-245467, have been submitted.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM5-3841)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

MURPHY BREEDING LABORATORIES, INC. (NIH-75-C-579)

This contract furnishes approximately 312,000 six-week-old $CD2F_1$ (BALB/c♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

NATIONAL ACADEMY OF SCIENCES (NO1-CM5-3850)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals; standards for nomenclature used to identify stocks and strains of laboratory animals; standards for methodology in maintaining axenic and gnotobiotic animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by ad hoc committees whose memberships represent commercial animal production colonies, governmental and academic institutions, commercial laboratories and non-profit research institutions.

NATIONAL ACADEMY OF SCIENCES (NO1-CM5-7013)

This contract Task Order provides for conferences on diverse subjects that are intimately associated with the field of biomedical research. The agenda for specific meetings may be concerned with a common problem, or to develop the basis for a research program that is being considered by a segment of the scientific community. Conferences in the past have been held on research facilities; diseases of laboratory animals; professional training; the provision of animals for biological models for research; gnotobiotic technology; and requirements for standards in specific areas. Speakers are invited to present their thoughts and time is provided for open forum discussions. The proceedings of these conferences are published and made available to the scientific community.

NATIONAL NAVAL MEDICAL CENTER (YO1-CM5-0100)

This interagency agreement provides support to the Hematology-Oncology Section of the Bethesda Naval Medical Center. The agreement is designed to integrate the breast cancer studies of the Bethesda Naval Medical Center and the National Cancer Institute's Medical Breast Cancer Section. Joint therapeutic protocols for patients with primary and metastatic disease are in the program. Patient accrual to the studies has been satisfactory.

NAVAL WEAPONS CENTER (YO1-CM5-0101)

This project will develop and evaluate prototype instruments for the quantitation of growth in granulocyte precursor cells. The monitoring technique closely follows that developed in missile guidance systems for remote detection of small targets against complex bright backgrounds. The National Cancer Institute and National Heart and Lung Institute laboratories are collaborating

NAVAL WEAPONS CENTER (YO1-CM5-0101) (CONTINUED)

in the design and evaluation of three prototype instruments for ongoing Division of Cancer Treatment studies of leukopoietic mechanisms, the assessment of abnormalities in white cell proliferation, and the determination of marrow function associated with other diseases.

NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC. (NO1-CM0-2035)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The Contractor carries out the service by: (1) performing iso-antigenic typing tests of tumors used in the various screening tests, enabling Drug Research and Development Program to maintain and distribute tumors identical to the prototype; (2) developing techniques and material for antigenic typing of tumors new to the screening program; (3) determining strain specificity of tumors originating in the inbred mouse; and (4) performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers.

NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC. (NO1-CM3-3726)

The objective of this contract is the development of antiviral-antitumor agents from a class of ansa macrolide compounds, the streptovaricin complex. These agents were first shown by the Principal Investigator to be reverse transcriptase inhibitors and to interfere with viral replication. A significant finding has been their later observation of relatively selective in vivo effects, i.e., minimum cytotoxicity yet interference with viral replication. Streptovaricin complex fraction has been isolated and shown to inhibit spleen focus forming virus component of Friend virus at very low concentrations. Attempts are underway to isolate large quantities of this component to determine its structure and to carry out acute toxicity studies.

NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC. (NO1-CM3-3734)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year new drugs and new drug combinations have been explored in patients with distant metastases.

NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC. (NO1-CM4-3714)

This contract is designed to undertake combined modality clinical studies in testicular tumors. The initial protocol treats Stage II nonseminomatous lesions with surgery followed by random allocation to radiotherapy, chemotherapy or radiotherapy plus chemotherapy.

NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC. (NO1-CM4-3782)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC. (NO1-CM4-3785)

The objective of this project is to develop a new test system, Profile of Drug Interactions. The approach to be used involves a determination in cell culture of the types of interaction (i.e., synergistic, additive or antagonistic) between the new drug and a selected panel of known chemotherapeutic agents or metabolic effectors. The interaction profile pattern produced can then be compared with those of other antitumor drugs to detect similarities in mechanisms of action. This system has successfully identified a group of structurally dissimilar inhibitors of ribonucleotide reductase as having similar biochemical actions.

NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC. (NO1-CM4-3794)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC. (NO1-CM5-3834)

This contract is designed to support prospective randomized, controlled studies using combination chemotherapy in the treatment of breast cancer.

NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC. (NO1-CM5-7034)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

NEW YORK STATE DEPARTMENT OF HEALTH & HEALTH RESEARCH, INC. (NO1-CM5-7039)

The objective of this contract is to establish *in vivo* and *in vitro* immunoassay systems that would be used to test the selective effects that various anticancer drugs may have on the immune response. The studies include evaluation of the immunosuppressive activity of antineoplastic agents in order to aid in the selection of candidate drugs; evaluation of the immunostimulating properties of various agents for possible application in cancer therapy; and determination of the effect of drugs on the antigenic properties of normal and tumor tissues in order to assess any influence on chemotherapeutic response.

NEW YORK UNIVERSITY MEDICAL CENTER (NO1-CM6-7059) (FORMERLY NO1-CM7-1174)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

NORTHEASTERN UNIVERSITY (NO1-CM5-3741)

The primary objective of this contract is the development of CNS-penetrating antineoplastic agents. The development of such agents is of importance both for the treatment of primary CNS neoplasms and for destroying lesions which are metastatic to the brain. Two groups of compounds, aminoisoquinolines and aporphines, are being synthesized as the brain-penetrating portion of the molecule. These kinds of structures are incorporated with alkylating agents to utilize the known effectiveness of alkylating agents against a variety of human malignancies. To date, most of the compounds prepared have been modifications of aminoisoquinolines. The compounds submitted are of a high degree of purity and in quantity sufficient for the complete testing protocol. Future work will include the synthesis of various aporphines with alkylating functions.

NOTRE DAME, UNIVERSITY OF (NO1-CM2-3718)

Leukemia patients under treatment by chemotherapy are highly vulnerable to infection. Microorganisms that are part of the normal human flora may themselves cause serious infection. During the course of investigations under this contract, AKR mice have been successfully decontaminated and remained so when maintained under sterile conditions. These mice, when treated with immunosuppressive drugs, survived just as readily as do germ-free animals.

OHIO STATE UNIVERSITY (NO1-CM5-3828)

The purpose of this contract is to obtain information on the disposition and metabolism of new agents entering Phase I clinical trial. The Contractor has begun investigation of the metabolism and disposition of cytembena in experimental animals and has developed methods for determination of parent compound and several metabolites. Studies in man using ^{14}C-labeled compound are planned in the coming year and the compound has been synthesized.

OHIO STATE UNIVERSITY (NO1-CM5-7015)

This contract is providing one of the two necessary components for the synthesis of daunomycin and adriamycin analogs. The immediate objective is to synthesize fraudulent sugars related to the sugar, daunosamine, an integral part of the

OHIO STATE UNIVERSITY (NO1-CM5-7015) (CONTINUED)

daunomycin and adriamycin molecules. The design and synthesis of these sugars and their coupling to selected aglycones is part of an overall plan to find anthracycline antitumor agents with less cardiac toxicity.

OHIO STATE UNIVERSITY (NO1-CM6-7060) (FORMERLY NO1-CM7-1175)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM3-3736)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year, new drugs and new drug combinations have been explored in patients with distant metastases.

OREGON STATE UNIVERSITY (NO1-CM2-3201)

The purpose of this contract is to acquire information on the disposition of drugs in animals so that such information is available prior to initiating clinical trials. Specifically, the three nitrosoureas, BCNU, CCNU and MeCCNU are being studied from two points of view. First, the complicated metabolic alterations these drugs undergo are being studied in detail. Second, the effects of these agents on the ability of the host hepatic microsomes to metabolize other drugs is under investigation.

PARKE, DAVIS & COMPANY (NO1-CM2-3708)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 50% of the effort on this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

PARKE, DAVIS & COMPANY (NO1-CM4-3754)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermentation beers from various microbes isolated from unique substrates from various parts of the world and fermented under different environmental and stress situations; (2) an *in vitro* tissue culture assay laboratory which will assist in pre-screening fermentation broths for cytotoxicity and will be used to help assay chemical fraction, fermentation improvement samples and large pilot plant lots more quickly; (3) a small biotransformation program; (4) the isolation work required to obtain the active component from the confirmed active beers; (5) the production of large quantities of antineoplastic agents approved for clinical trials; and (6) the preparation of appropriate dosage forms of such agents.

PENNSYLVANIA, UNIVERSITY OF (NO1-CM3-3719)

This contract provides, on a multiphase basis, for carrying out the design and installation of the Chemical Information System to handle a wide range of compound accessioning activities. This new system provides improved capability to: (1) identify which compounds are new to the Drug Research and Development Program, including the flagging of analogs and duplicates of compounds of interest; (2) produce computer-generated shipping documents and other communications (including chemical structural diagrams) to suppliers, screeners, in-house staff and contractors engaged in the acquisition, storage and distribution process; (3) maintain inventory control; (4) conduct on-line searches involving both structural and nonstructural information, using simplified inquiry procedures and providing structure output; and (5) maintain management and operational control of the system. The system has been installed in the Chemical Abstracts Service (CAS) which is the Contractor operating the system. The final phase of this contract provides for completing the system documentation and training CAS programmers to assume the responsibility for system maintenance.

PENNSYLVANIA, UNIVERSITY OF (NO1-CM5-3830)

This contract is an outgrowth of a previous contract (NO1-CM1-2188). The object is to evaluate the acute and chronic effects of selected antitumor drugs on the energy performance status of isolated, perfused organs using both surface fluorescent and transmitted light absorbance methods developed by the Contractor. These models are to be applied to delineating the biochemical basis of either the therapeutic activity or limiting toxicity of selected drugs and their analogs.

PENNWALT CORPORATION (NO1-CM1-2309)

The synthetic effort on this contract terminated at the end of Fiscal Year 1975. Prior to completion of the synthetic work, many coordination compounds of platinum and rhodium were prepared and tested. The ligands included

PENNWALT CORPORATION (NO1-CM1-2309) (CONTINUED)

pyridine, meta and para substituted pyridines, dimethylsulfoxide, glycine, thiocyanate, diethylenetetramine, tetraazacyclododecane, and hexaoxacyclotetradecane. None of the compounds exhibited sufficient antitumor activity to advance to the next screening stage. During the terminal period of this contract, effort will be directed towards evaluating the testing data and studying the structure-activity relationship.

PFIZER, INC. (NO1-CM5-3805)

The main purpose of this contract is to produce large quantities of primate and putative human type-C viruses in tissue culture and to purify and concentrate these viruses from the tissue culture fluids. In addition, the Contractor is to prepare certain viral components both to use in quality control of virus production and to develop radioimmunoassay techniques used in the investigation of human neoplasms.

PHARM-ECO (NO1-CM2-3707)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 70% of the effort of this contract is devoted to the preparation of large quantities of material, in the multi-kilogram range.

PHILIPS ROXANE LABORATORIES, INC. (NO1-CM1-2139)

This service contract provides the Division of Cancer Treatment with facilities for development, formulation and production of oral dosage forms of investigational drugs. The dosage forms prepared at Philips Roxane Laboratories are packaged, labeled and shipped to the National Cancer Institute for subsequent distribution to clinical investigators.

POMONA COLLEGE (NO1-CM3-3703)

The contract with Pomona College has two objectives: (1) the application of regression analysis for the discovery of relationships between the structure-related parameters of potential anticancer agents and their antitumor activity, and (2) an investigation of the molecular basis of drug action in enzyme inhibition systems. The Contractor is currently investigating the structure-activity relationships among analogs of adriamycin, thio-TEPA, 5-FU, 6-MP and thioguanine and he has measured partition coefficients for compounds being studied in the intramural program (aziridinyl quinones and terephthalanilides). The Contractor consults with synthetic contractors and suggests analogs for synthesis based on structure-activity principles. The investigation of structural factors affecting structure-activity relationships in enzyme-

POMONA COLLEGE (NO1-CM3-3703) (CONTINUED)

substrate systems has continued. Emphasis was placed on the study of inhibitors of dihydrofolate reductase. A method for total assay of DHFR in an inert atmosphere has been developed. This contract effort is being recompeted this Fiscal Year.

PUBLIC HEALTH SERVICE, DIVISION OF FEDERAL HEALTH PUBLIC SERVICES (TRANSFER OF FUNDS - YO1-CM4-0003) (BALTIMORE, MARYLAND)

This Transfer of Funds permits service support of the National Cancer Institute, Intramural Baltimore Cancer Research Center program. This includes suitable laboratory facilities, support personnel and other administrative support.

RADIOBIOLOGICAL INSTITUTE (NO1-CM5-3763)

The objective of this contract is to conduct preclinical investigations of the combination modality of radiation and hypoxic radiosensitizers plus chemotherapy. Appropriate tumor test models are being explored and developed for this combination modality. With such models determination will be made of combinations and regimens of drug plus radiation that are optimal. The program is designed to provide an appropriate preclinical basis for the clinical application of radiation plus chemotherapy.

RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK (NO1-CM3-3715)

This contract is designed to support the statistical and administrative efforts of the cooperative protocol studies of the Working Party for Lung Cancer.

RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK (NO1-CM5-3766)

This contract is to perform *in vivo* and *in vitro* basic investigation and development of new experimental models for studying the effects of anticancer agents on immune responses. Specifically the effects on T and B cells, macrophages and the Hh systems will be assessed.

RESEARCH TRIANGLE INSTITUTE (NO1-CM9-2019)

The primary purpose of this contract is to fractionate confirmed active plant extracts in an attempt to isolate and identify the active antineoplastic compound. In addition, active fermentation materials obtained from other contractors are isolated, purified and identified. The plants used for this work are primarily obtained from the U. S. Department of Agriculture. The Contractor has many plants which are or have been in some stage of fractionation and several crude fermentation materials which are being purified. Several of these active agents have been isolated and are being evaluated more broadly. A KB cell culture facility is used to expedite fractionation work.

RHODE ISLAND, UNIVERSITY OF (NO1-CM4-3778)

This contract provides for the synthesis of nitrogen heterocyclic compounds with potential anticancer properties. The compounds being synthesized are analogs and derivatives of the pyrazoline and triazole ring system. The compounds have the potential to undergo a unique fission to produce metabolically active species.

SIDNEY FARBER CANCER CENTER (NO1-CM4-3781)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced, surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

SIDNEY FARBER CANCER CENTER (NO1-CM4-3799)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

SIDNEY FARBER CANCER CENTER (NO1-CM5-3839)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I studies on dianhydrogalactitol, NSC-132313 have been completed. Phase I studies on high dose MTX thymidine rescue are proceeding. Protocols for Phase I studies with maytansine, NSC-153858 and neocarzinostatin, NSC-157365, have been accepted.

SIDNEY FARBER CANCER CENTER (NO1-CM5-7035)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

SIMONSEN LABORATORIES, INC. (NO1-CM5-3817)

The Contractor maintains a genetic production colony of seven inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production colonies.

SIMONSEN LABORATORIES, INC. (NO1-CM5-3847)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SIMONSEN LABORATORIES, INC. (NIH-75-C-578)

This contract furnishes approximately 312,000 six-week-old $B6D2F_1$ (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CM5-3820)

The object of this contract is to extend the techniques of *in situ* molecular hybridization to the presence of oncornavirus nucleic acid sequences in individual mammalian cells. To this end tumor virus nucleic acids labeled with ^{125}I to the highest possible specific activity are being tested for their usefulness for *in situ* hybridization.

SMALL BUSINESS ADMINISTRATION (NO1-CM4-3719)

The purposes of this contract are to provide supportive services in immunology, virology and tissue culture to the cancer chemotherapy program. The immediate goals are: (1) to develop a sensitive *in situ* immunological assay system for detecting virus-related antigens in human leukemic cells as a potential diagnostic tool; (2) to perform other immunological tests including immunofluorescent tests and complement fixation tests for virus-related antigens in human leukemic cells and other pertinent cells; (3) to test various compounds available to the Laboratory of Tumor Cell Biology investigators for their inhibitory effect on type-C virus biological activity and on human leukemic cells *in vitro*; (4) to perform other specialized supportive procedures essential to LTCB investigators, including cytogenetic analyses, cell cloning, and virological procedures of biological interest. This work is carried out at Biotech Research Laboratories.

SOUTHERN ANIMAL FARMS (NO1-CM5-3845)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies.

SOUTHERN ANIMAL FARMS (NIH-75-C-599)

This contract furnishes approximately 312,000 CD2F_1 (BALB/c♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CM2-3241)

This contract was instituted to define the pharmacokinetics of adriamycin and guanazole in man. Initial efforts have defined the metabolism and distribution of adriamycin in rabbits, and a theoretical model, based on the findings in the rabbit, is currently being applied to the clinical situation. In the past year pharmacokinetic studies have been performed on approximately 20 patients and the results are being correlated with the existing animal model for drug disposition. This contract will be re-competed in Fiscal Year 1977.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CM4-3795)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CM5-3842)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced, surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-3756)

The objective of this multifaceted contract is to apply fundamental biological principles to develop new and improved *in vivo* and *in vitro* laboratory models designed to aid the Division of Cancer Treatment in the discovery of more effective anticancer therapies, individual drugs, combinations of drugs, combined treatment modalities, and the optimal conditions for their use. Success of the effort rests on the ability of the Contractor to respond to Division of Cancer Treatment's needs utilizing test systems of established value; but to a larger extent, to comprehend program needs relative to the development of new and improved biological testing systems; and to respond by conceiving, developing, and using such systems. Tasks conducted may be categorized broadly as (1) service-type testing in response to instructions from the National Cancer Institute and in accord with established National Cancer Institute drug screening protocols, (2) detailed studies for which experimental approaches are indicated in more or less detail by the National Cancer Institute, (3) developmental studies indicated as desirable by the

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-3756) (CONTINUED)

National Cancer Institute, but for which methodologies and approaches are expected to be formulated and tested by the Contractor, and (4) exploratory basic studies aimed at the realization of the Division of Cancer Treatment objectives.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-3762)

Southern Research Institute is one of the basic synthesis contractors for the Drug Research and Development Program. The chemistry program is oriented to the biological and biochemical rationales developed in other programs, and is integrated with the screening, therapeutic and biochemical activities at Southern Research Institute. The areas of investigation include: purine antimetabolites, including 9-substituted-9H-purines, ring analogs of purines, nucleotides and nucleosides; folic acid antagonists, directed toward the preparation of "simpler" derivatives and variants of the methotrexate and aminopterin structures; imidazole antimetabolites and analogs of imidazoles; streptozotocin analogs; aryl and heterocyclic triazenes; metabolites of cytoxan; and compounds containing very reactive chemical moieties.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-3780)

The purpose of this contract is to obtain basic quantitative pharmacologic information in animals which will indicate the requirements of drug dosage and scheduling necessary to give maximum tumor cell kill while minimizing toxicity to the host's normal cells. This involves the development and application of highly sensitive analytical methodology to measure drugs and their metabolites in body fluids and tissues and the estimation of half-lives and cumulative Cxt values in animals and man. During the past year, studies have involved cyclocytidine, arabinosylcytosine, actinomycin-D, indicine-N-oxide and hexamethylmelamine.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-3784)

The major objective of this contract is to develop basic biochemical knowledge useful in the design of new drugs or in the more effective use of known agents. To accomplish this aim, projects are underway on (1) determining if new antitumor drugs have biochemical activities similar to those of clinically established drugs, (2) providing clear leads to biologically significant biochemical properties of new antitumor drugs, (3) evaluating the molecular mechanisms of action of new antitumor drugs, (4) developing systems to allow selection of structural analogs of clinically established antitumor drugs which have more desirable biochemical properties, and (5) developing biochemical rationales for the combined use of clinically established antitumor drugs.

SOUTHERN RESEARCH INSTITUTE (NO1-CM5-7000)

The principal objective of this contract is to compare the relative toxicity and therapeutic activities of cancer chemotherapeutic agents alone and in combination when different dosage regimens are used in rodent tumor systems and in non-human primates or dogs. These studies are intended to serve as a basis for the design of a combination drug regimen acceptable for clinical trial. The combinations of adriamycin-cyclophosphamide and 5-fluorouracil-methyl CCNU are currently under study.

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CM6-7075)

This is a service contract for the in vitro testing of new synthetic and natural product materials. The major portion of the test capacity is utilized for the natural products program, which includes the testing of crude materials and for the bioassay of natural product fractions that show activity both in vivo and in vitro. Testing is carried out at a level of approximately 5,000 tests per year according to Drug Research and Development Program protocols.

SPARTAN RESEARCH ANIMALS, INC. (NIH-75-C-582)

This procurement contract provides for the supply of 156,000 CD2F_1 (BALB/c♀ x DBA/2♂) hybrid mice for the Drug Research and Development Program compound evaluation studies. Breeding animals originate in genetic centers.

ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE (NO1-CM5-7020)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

STANFORD RESEARCH INSTITUTE (NO1-CM1-2162)

This service preparative contract provides for the synthesis of radiolabeled chemicals and drugs for use in preclinical pharmacologic and clinical studies. Many of the materials prepared are not available from commercial sources and are synthesized. All materials, whether prepared by the contract group or acquired from other sources, are analyzed for purity and identity by radio-autography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

STANFORD RESEARCH INSTITUTE (NO1-CM2-3200)

The purpose of this contract was to acquire information on the disposition of drugs in animals so that such information was available prior to initiating clinical trials. The following investigations were conducted: (1) plasma levels and urinary, biliary and fecal excretion rates of drugs and/or metabolites of compounds of interest to the chemotherapy program; (2) tissue distribution of the parent drug and metabolites after oral and parenteral administration; (3) isolation and identification of the major drug metabolites; and (4) properties of the enzyme systems responsible for metabolism (subcellular localization, cofactor requirements and susceptibility to known inducers and inhibitors of drug metabolism). Both *in vivo* and *in vitro* studies were conducted using at least three animal species, including one species of subhuman primates. This contract terminated March 1976.

STANFORD RESEARCH INSTITUTE (NO1-CM3-3723)

This contract is concerned with quality control studies on compounds prepared for preclinical toxicology and clinical trial. The Contractor evaluates bulk chemicals and formulation drugs as to their identity, assay and purity, to provide assurance of high quality and the necessary analytical data for the Food and Drug Administration. This area of work provides elemental analysis, as well as the more sophisticated techniques such as chromatography (paper, thin layer, liquid and gas), absorption spectroscopy (ultraviolet, visible and infrared), nuclear magnetic reasonance, and other physical-chemical procedures appropriate for the situation. In some cases, methods of assay must be developed for new structures in order to maintain adequate surveillance on the quality of all drugs used in clinical trials.

STANFORD RESEARCH INSTITUTE (NO1-CM3-3742)

Stanford Research Institute is one of the three major contractors devoted to the synthesis of new chemical structures. The research program encompasses such areas as nucleosides, antimetabolites, antibiotics, alkaloids and other areas as may be requested.

Special attention is being directed to the study of the chemistry of the antibiotics, daunomycin and adriamycin, in order to develop agents of greater specificity and lower toxicity. Analogs of daunomycin and adriamycin are being prepared. A modest effort is being extended toward preparing semi-synthetic and simplified synthetic adriamycin analogs that retain only major features of the parent molecule. Also under investigation, is the synthesis of a group of compounds designed to intercalate into DNA in the manner proposed for several antitumor compounds and some unusual heterocyclics. Small efforts are directed towards preparation of analogs of ethidium bromide, 2,3-dihydro-imidazo[1,2-b]pyrazole, formycin A and B, the indole alkaloid, ellipticine, and the antikinetic, piperazinedione.

STARKS ASSOCIATES, INC. (NO1-CM2-3203)

This service preparative contract supplies the Drug Development Branch with compounds required for drug evaluation studies, with 50% of the effort being devoted to the production of clinical materials. Preparations vary in quantity from 5 grams to multikilograms. The compounds prepared are not readily available in the quantities required on the open market or from the original supplier.

STARKS ASSOCIATES, INC. (NO1-CM5-3769)

This service contract is for the resynthesis of compounds not readily available from commercial sources or from the original supplier in the amounts needed. The compounds prepared are for the completion of definitive testing or for clinical or preclinical evaluation. About 25% of the effort of this contract is devoted to the preparation of large quantities of materials. Many of the preparations involve multistep sequences.

STARKS, C. P., INC. (NO1-CM5-3745)

This service contract supports the Drug Development Branch's efforts in the acquisition of chemicals and drugs for evaluation in the primary screen. The Contractor is required to: (1) provide liaison services to develop new sources of material and to encourage potential suppliers to participate in the program through voluntary contribution of samples; (2) collect samples; (3) provide support services in the processing of compounds; (4) aid in the management of computer-generated reports relating to compound acquisitions; and (5) assist in the development of methods for selecting materials for the screen.

TACONIC FARMS, INC. (NIH-75-C-597)

This contract furnishes approximately 260,000 B6D2F_1 (C57BL/6♀ x DBA/2♂) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies.

TENNESSEE, UNIVERSITY OF (NO1-CM0-2084)

The objectives of this contract mainly include formulation development studies and small-batch production of oral, solid dosage forms. The Contractor also has the capabilities to prepare parenteral dosage forms in pilot-batch lots. This contract will be terminated during this contract period.

TENNESSEE, UNIVERSITY OF (NO1-CM5-7021)

The purpose of this contract is to provide clinical data from selected patients with diagnosed brain tumors in order to evaluate the efficacy of therapy. Both single agent treatment and combined modalities therapy are included in

TENNESSEE, UNIVERSITY OF (NO1-CM5-7021) (CONTINUED)

accordance with the protocols of the Brain Tumor Study Group. The acquisition of patients into this prospective controlled, randomized study permits a careful analysis of comparative clinical course and survival for experimental and control groups.

TEXAS INBRED MICE COMPANY (NO1-CM5-3816)

This genetic production center supplies a variety of inbred and hybrid rodents for tumor transplantation, for hybrid production, and for compound evaluation studies. Animals are supplied from a colony of 11 strains of rodents.

TEXAS, UNIVERSITY OF (NO1-CM5-3832)

This contract is designed to investigate the role of protected environments and prophylactic antibiotics as adjuvants to the remission induction chemotherapy of non-Hodgkin's lymphoma and metastatic soft-tissue sarcomas in adults.

TEXAS, UNIVERSITY OF (HEALTH SCIENCES CENTER AT DALLAS) (NO1-CM5-3767)

This contract has been designed to develop sublines of neuroblastoma and astrocytoma cells in tissue culture which are resistant to antitumor agents of interest in the drug development program. Resistant sublines will be examined with respect to mechanism of resistance and cross-resistance to other antitumor agents.

TEXAS, UNIVERSITY OF (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM3-3710)

The objective of this contract is to identify clinical effectiveness of antitumor agents for the treatment of advanced ovarian carcinoma. This common tumor of women is known to be quite sensitive to a variety of alkylating agents as a class. The Contractor will randomize 100 previously untreated patients with Stage II or IV ovarian carcinoma to one of three choices of treatment. Melphalan, 5-FU and hexamethylmelamine studies are nearly completed. All three drugs show significant activity.

TEXAS, UNIVERSITY OF (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM5-3773)

The purpose of this contract is to carry out studies on the cellular and molecular pharmacology and physiological disposition of new antitumor agents under development by the Division of Cancer Treatment, National Cancer Institute. During the current year, studies have been performed on aspects of the pharmacology of the following agents: Baker's antifol, (2-chloro-4-(4,6-diamino-2,2-dimethyl-s-triazine-1-(2H)-yl) phenoxy)-N,N-dimethyl-m-

TEXAS, UNIVERSITY OF (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM5-3773) (CONTINUED)

toluamide ethanesulfonate, NSC-139105); Asaley (ethyl-N-acetyl-DL-sarcolysin-L-leucinate, NSC-167780); DIC (5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide, NSC-45388); cyclocytidine (O^2,2'anhydro-1-β-D-arabinofuranosyl-cytidine, NSC-145668) and the Yoshi dimethanesulfonate compound, 3,3'-iminodipropyl dimethanesulfonate, NSC-102627). Studies have been initiated on the effect of the immunoadjuvant derived from Corynebacterium parvum on hepatic drug-metabolizing microsomal enzymes. A cellular pharmacology program has been initiated, utilizing human melanoma and lymphoma and Chinese hamster ovary cell lines. The effects of antitumor agents of current therapeutic interest are evaluated in terms of cell cycle stage sensitivity, cell kill as a function of duration of treatment, and repair of drug-induced injury. Actions of actinomycin D, adriamycin and bleomycin on chromosomal morphology have been studied. Sixteen publications have appeared during the past year describing research carried out under the auspices of the contract. Studies under this contract are performed primarily in experimental animals and in cell culture systems, but are closely interrelated with Phase I clinical trials and clinical pharmacology studies conducted at the M. D. Anderson Hospital and Tumor Institute and elsewhere.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM3-3737)

This contract has been designed to conduct eradicative clinical trials of patients with bronchogenic carcinoma, explore new and promising avenues of therapy for lung cancer, and devise and implement therapeutic protocols that will lead to a systematic and progressive understanding of the disease and promote its control. During the past contract year, new drugs and new drug combinations have been explored in patients with distant metastases.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM4-3786)

The major emphasis of this project is to provide a detailed evaluation of the effects of new antitumor drugs on DNA biosynthesis or function, RNA synthesis, processing or function (ribosomal, messenger, and transfer RNA) and incorporation into RNA or DNA.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM4-3801)

This contract was designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment, National Cancer Institute or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. Phase I clinical studies with diglycoaldehyde, NSC-118994; asaley, NSC-167780; piperazinedione, NSC-135758; ftorafur,

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM4-3801) (CONTINUED)

NSC-148958; and Baker's antifol, NSC-139105, have been completed. Protocols for clinical studies with hycanthone, NSC-134434; maytansine, NSC-153858; and deazauridine, NSC-126849; have been accepted.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM5-7042)

This contract is designed to support Phase II-III studies in solid tumors, by means of prospective randomized, controlled clinical trials involving the use of combined modalities.

TULANE UNIVERSITY (NO1-CM5-3815)

This primary genetic center serves to establish foundation colonies of caesarian-derived inbred rats (strains M520, OM/N, Fischer 344, Buffalo, W/f., ALB, ACI and other inbred strains as required). Its objectives are to improve the quality and maintain the genetic characteristics of the strains for program activities. Foundation colonies are propagated in germ-free isolators, and small-scale production colonies are housed in bio-containment environments.

UPJOHN COMPANY (NO1-CM4-3753)

This contract has for its primary objective the research and development of antineoplastic agents from fungi, bacteria and actinomycetes fermentation beers, including isolation, purification, characterization, production, biochemistry and pharmacology efforts.

The Contractor is involved in the development and exploitation of cell culture methods, both for assay on fractionation samples and for primary screening. Leads developed from this screen are being tested in-house in the leukemia in vivo screen and actives are given top priority for chemical fractionation.

Major efforts in this contract are also devoted to biotransformation of known Upjohn antibiotics, studies on the mechanism of action and physiological disposition of clinical drugs developed within the contract program, and phases of toxicological and pharmacological studies within the capability of the Contractor and requested by the National Cancer Institute.

UTAH, UNIVERSITY OF (NO1-CM4-3806)

This contract is for the synthesis of nucleoside analogs and derivatives, including nucleoside antibiotics, for screening as antitumor agents. The compounds selected for synthesis are based upon biological and biochemical rationale. The areas of investigation include: (1) analogs of the antibiotics, tubercidin, sangivamycin and toyocamycin and other nucleoside

UTAH, UNIVERSITY OF (NO1-CM4-3806) (CONTINUED)

antibiotics, (2) derivatives of the C-nucleoside formycin, (3) pyrazolo [4,3-d]pyrimidine and pyrazolo[3,4-d]pyrimidine nucleosides, (4) imidazo [4,5-c]pyridine, imidazo[4,5-b]pyridine and v-triazolo[4,5-c]pyridine nucleosides, and (5) imidazole and benzimidazole nucleosides. This Contractor provides these difficult materials in quantities sufficient for in vivo anti-tumor evaluation by methods of synthesis developed by this group.

UTAH, UNIVERSITY OF (NO1-CM5-3827)

The purpose of this project is to determine the pathologic effects of the combination of radiation and adriamycin therapy using the New Zealand white rabbit as a model. It has been well documented that ionizing radiation can produce cardiac damage in both humans and in the New Zealand white rabbit. Similarly, adriamycin has been shown to produce cardiomyopathies in both humans and the New Zealand white rabbit. With the increased use of adriamycin in treating a variety of human malignancies, particularly when combined with radiation, the question is raised of the possibility of enhanced cardiac damage in radiation or drug dose ranges which have been previously considered to be safe.

VALUE ENGINEERING COMPANY (NO1-CM3-3706)

Data processing services are provided by this contract to the Drug Research and Development Program. The main areas of emphasis are: (1) reducing and disseminating information developed in the screening program of the Drug Evaluation Branch, Drug Research and Development Program (including special queries of the master files via computer); (2) documenting all programs and contractor's procedures for data handling and running computer programs; (3) maintaining the computer programs so that they are able to run in the Division of Computer Research and Technology environment; (4) reducing and disseminating information on the receipt of compounds including those of particular interest to the Division of Cancer Treatment program; (5) expanding the present system to incorporate secondary testing not previously stored on the master files; (6) redesigning the existing system and developing new formats and methods of data collection; (7) providing instructions for screening laboratories and suppliers of materials relating to collection and dissemination of data; and (8) participating in scientific meetings.

VALUE ENGINEERING COMPANY (NO1-CM5-3821)

This contract provides for (1) programming support for the Drug Distribution and Inventory System and (2) technical support for maintaining associated data files. These computer files serve as a record of all drugs sent from the National Cancer Institute to clinical investigators, and include all pertinent drug, investigator and protocol information. In the past year a drug order verification system was developed. Under this system all drug orders are objectively verified against protocol requirements. Increased automation will be added to this system in the coming year.

VERMONT, UNIVERSITY OF (NO1-CM3-3716)

This contract provides for the extension of the Computerized Problem-Oriented Medical Information System to the Division of Cancer Treatment by integrating structured input ("cancer displays") into the system, thereby coupling learning with doing for all personnel who care for individual cancer patients entered on problem-specific plans (research protocols).

VETERANS ADMINISTRATION (YO1-CM4-0004)

This contract includes support for the NCI-VA Medical Oncology unit, the Veterans Administration Lung Group, and the Veterans Administration Surgical Adjuvant Group. The first is a 30-bed oncology unit devoted to clinical protocol work in lung cancer, prostate cancer, and other solid tumors. Investigations include new approaches in diagnosis and therapy requiring special in-patient facilities. The Veterans Administration Lung Group is concerned with providing data on treatment of advanced lung cancer with chemotherapy, immunotherapy and radiation. The Group represents a stable resource of patients treated for lung cancer according to randomized protocol studies. The Veterans Administration Surgical Adjuvant Group is similarly made up of a network of Veterans Administration Hospital investigators exploring the usefulness of chemotherapy and immunotherapy and radiation as adjunct to surgery, by utilizing randomized patient studies.

VETERANS ADMINISTRATION HOSPITAL (SOUTH CAROLINA) (YO1-CM6-0102)

The Contractor is to conduct research on and synthesize metal coordination compounds as potential carcinostatic agents. Present emphasis is on the synthesis of platinum coordination compounds which are analogs of the clinically useful diammine dichloro platinum complex. The main aim of the investigation is the design and synthesis of platinum complexes having improved water solubility and less toxicity.

VIRGINIA, UNIVERSITY OF (NO1-CM1-2099)

The major objective of this contract is the fractionation of confirmed active plants in order to isolate in a pure state and identify the antitumor active components. Plant materials used in this work are obtained, for the most part, through the U. S. Department of Agriculture.

WALTER REED ARMY MEDICAL CENTER (YO1-CM4-0005)

The primary objective of this Interagency Agreement is to provide support for Walter Reed Army Medical Center, Hematology-Oncology Service. This includes collaborative studies with Acute Leukemia Group B and the Clinical Oncology Program. Protocols are available for patients with acute lymphocytic leukemia, acute granulocytic leukemia, chronic lymphocytic and chronic granulocytic leukemia, Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, and brain, head and neck, breast and testicular tumors.

WARF INSTITUTE, INC. (NO1-CM4-3707)

This represents a service contract to prepare extracts of plant materials for screening by the screening laboratories. A semi-quantitative test for alkaloids is run on plant materials of sufficient interest to be considered for fractionation. The majority of the Contractor's plants are obtained through a contract with the U. S. Department of Agriculture. During the year, the special study of the extraction procedure was continued involving the preliminary fractionation, on a trial basis, of 500 plants in order to develop a procedure for increasing the yield of P388-active plants. This study was successful and a new extraction procedure was adopted.

WARF INSTITUTE, INC. (NO1-CM5-3770)

This is a service contract for the in vivo screening of new crude plant extracts, fractionations of previously confirmed natural products, new synthetic materials, and follow-up testing of active materials. Currently, testing is being conducted at a level of approximately 50,000 tests per year according to Drug Research and Development Program protocols. The P388 lymphocytic leukemia is employed as the model for the initial testing of all materials. Other Drug Research and Development Program tumor systems are used in the further testing of active materials or for analogs of known materials. Schedule dependency studies are conducted, upon request, for materials of potential clinical interest. Methodology and/or research and development, as well as special research programs, are scheduled as the need arises and are directed by the Project Officer.

WASHINGTON HOSPITAL CENTER (NO1-CM4-3710)

The objectives of this contract were to label bleomycin with gamma-emitting isotopes for external counting, and to study the tumor localizing properties of this material for staging and diagnostic evaluation. Material was shown, in a preliminary manner, to localize in a higher proportion of tumors than other isotopes such as Gallium-67. The ability to identify tumor foci in the body allowed for more precise staging evaluation as well as for following changes in tumor size after treatment.

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE (NO1-CM4-3731)

The cytocidal effects of single drugs on normal hematopoietic stem cells and malignant cells (L1210, AKR and L cells) are quantitated by using a spleen colony or lung colony assay. Drug pairs are then evaluated for their effects on tumor and host cell survival. From these data principles for drug-pair sequence and dose intervals may be disclosed.

WISCONSIN, UNIVERSITY OF (NO1-CM1-2275)

The principal objective of this contract is to study the mechanism of replication of genetic information, contained by RNA-tumor viruses, as well

WISCONSIN, UNIVERSITY OF (NO1-CM1-2275) (CONTINUED)

as the mechanism of integration of this information into the host chromosome. To accomplish this objective, studies are in progress with purified viral enzymes in order to understand clearly the enzymic reactions involved. The results on each isolated reaction will be assessed with relation to the physiology and life cycle of the intact virus. The DNA and RNA enzymes have been implicated in the etiology of human cancer. An understanding of the properties and mechanisms of these enzymes is, therefore, fundamental for the eventual solution of this problem.

WISCONSIN, UNIVERSITY OF (NO1-CM6-7076) (FORMERLY NO1-CM3-3733)

This contract provides rapid cell culture cytotoxicity assays as an aid in isolation and purification of potential antitumor agents of plant origin. Standardized assays are performed in accordance with procedures described by Drug Research and Development Program protocols. Occasional methodology studies are carried out at the request of the Project Officer.

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CM3-3711)

The objective of this contract is to provide in vitro screening systems to select promising anticancer agents of the antifolate type. The compounds are assayed for inhibition of partially purified dihydrofolate reductase from L1210 leukemia cells, from human myelogenous leukemia and normal red blood cells. Special preclinical pharmacologic studies are carried out with selected agents, as is the gathering of appropriate data from patients receiving this type of drug.

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CM4-3797)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas.

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CM5-3824)

The purpose of this contract is to mount a chemotherapeutic attack, by means of new agents designed and prepared under this contract, on the neoplastic cell membrane, particularly on the synthesis of its glycoprotein components, aimed at either inhibition of membrane synthesis or alteration of functional aspects of the membrane such as antigenicity, cellular adhesiveness, and/or mobility, cell cycle determinants, or the state of cellular differentiation.

As new agents are synthesized, their impact on a suitable number of the above-mentioned biological effects is determined.

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CM5-3843)

This contract is designed to explore in prospective randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) adjuvant therapy of locally advanced, surgically incurable gastric carcinoma comparing single agent chemotherapy versus combination chemotherapy versus single agent plus radiotherapy.

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CM5-7029)

The objective of this contract is to produce new anticancer agents based on their ability to intercalate and bind to nucleic acids. This would interfere with strand separation, which might be expected to result in suppressed or faulty replication of DNA or RNA. Evaluation of the agents synthesized is first by physical measurements of binding strength, melting temperatures, then by studies at the molecular level of effects on the replication and processing of various nucleic acids, and finally by antitumor effects in vitro and in vivo.

YESHIVA UNIVERSITY, ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CM4-3787)

The major emphasis of this project is to provide a detailed evaluation of new antitumor drugs which affect protein synthesis. For drugs having significant effects on protein synthesis, detailed studies on messenger binding, initiation, translocation and peptide bond formation will be done. In addition, detailed studies on the mechanism of action of new antitumor drugs which may directly affect the structure of DNA are performed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06401-04 ODCM

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Data Processing Systems for Scientific and Management Information

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Gordon	Systems Analyst	OD DCT NCI
OTHER:	A. Kontaratos	Planning Officer	OD DCT NCI

COOPERATING UNITS (if any)

Data Management Branch, Division of Computer Research and Technology, NIH

LAB/BRANCH

SECTION
Office of the Director, Division of Cancer Treatment

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.2	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

The Cancer Treatment Program is stratified for purposes of monitoring, analyzing and planning the allocation of resources. A stratification form is used by DCT staff to collect, aggregate, and tabulate monthly budgetary information. This information is then evaluated periodically to determine the adequacy of funding for each R&D line item.

The raw data for this DCT budget management system is obtained by the automation of contract data input via RCB, NCI; direct operations data input via DFM and the NCI Budget Office; and cooperative group grants information via DCRRC, NCI. The stratification forms have been designed to capture Disease- and Modality- oriented budget information, which is then applied to the financial data. Differentiation between "Research" and "Support" is also indicated.

Computer programs have also been written to generate National Cancer Program approach category 6 reports via fixed relationships with the stratification. An effort is also being made to incorporate a "Keyword" potential in the DCT data file similar to that of Cancerline.

Project Description

Objectives: 1. To establish a complete DCT budget management system by the automation of contract data input via RCB, NCI; direct operations data input via DFM and the NCI Budget Office; and cooperative group grants information via DCRRC, NCI.

2. Revision of the DCT Stratification to provide comprehensive Disease-Oriented and Modality-Oriented budget information.

3. Establishment of a microfiche information system for archival and distribution purposes.

4. Coordination of data pertaining to computer oriented expenditures within the Division of Cancer Treatment.

Methods Employed: 1. Through the use of DMB, DCRT program generation software, a single file containing all DCT contracts (commitments, Schedules A&B), direct operations data, and grants data is updated monthly at the computer terminal. This update is via the DMB, DCRT UPDATE and EDIT system; the data is entered into the WYLBUR Reformat Generator (a LCLN) for conversion to the appropriate transaction format. This update is designed for use by nonprogrammers. All entries in the file are classified with respect to the DCT Stratification. Differentiation between "Research" and "Support" is also indicated.

2. New Stratification forms have been designed to capture additional Disease- and Modality-oriented budget information. Concurrently, programs have been written by DMB, DCRT to reinitiate the DCT data file by use of this new information. Contractural information procurement continues as before, with the IMX-generated linkage to the RCB, NCI contract management system for monthly updates. Cooperative Group grants information is now obtained from DCRRC, NCI via WYLBUR and from DRG via the IMPAC system.

3. Monthly reports are now distributed and stored on microfiche.

4. With the cooperation of the Administrative Officers of DCT, data pertaining to DCRT accounts for DCT is now coordinated by this office.

Major Findings: 1. Programs are being written by DMB, DCRT to generate reports by various combinations of the DCT Stratification elements applied to contract, direct operations, and grants data as well as by DCT organization. In particular, several new reports will soon be available for classification of the DCT budget by the "new" DCT Stratification. Emphasis will be placed on Disease- and Modality-oriented summary reports. DFM is now providing DCT with reports that were previously done manually by the Administrative Officers. DCRRC, NCI provides DCT with grants information in the form of a WYLBUR data set.

2. Regeneration of the DCT data file will allow for the incorporation of many new variables, which pertain to the Cooperative Group grants that have been transferred from DCRRC. Accordingly, many new budget reports may soon be generated upon request.

3. An effort will be made to incorporate a "Keyword" potential in the DCT data file similar to that of Cancerline.

4. The availability of contract and DCRT account data has enabled this office to formulate the annual ADP plan requested by the OD, NCI. Statistics pertaining to the Data Processing expenditures of DCT are now available via the "new" DCT Stratification as applied to the DCT budget.

Significance to the Program of the Institute: The computer system that is being developed with the assistance of DMB, DCRT is utilized to monitor and assess budget information related to segments of the DCT program and to more specific project areas both in the intramural areas and in the contract program. The reports generated by this system lead to a detailed description of the DCT research program.

Proposed Course: Possible modifications of the new stratification form to allow for the receipt of treatment data from other divisions of NCI is contemplated. These modifications will, in turn, require another large scale revision of the information system with the subsequent generation of new reports by DMB, DCRT. Programs will also be written to generate NCP approach category 6 reports via fixed relationships with the stratification.

Additional reports will also be prepared using the AUTOTAB system to show budget balance type information. This system, which is a software package leased by DCRT from CAPEX Corporation, facilitates the preparation of financial reports. The system was greatly enhanced this past year by the introduction of the external file capability. It is this capability, which will make it possible to link AUTOTAB programs to intermediate summary data generated by the DMB, DCRT programs for Stratification-oriented reports.

Automation of the retrieval of DCRT account data from DCRT tapes in the form required by the annual ADP plans will be investigated. This data combined with DCT stratification data will be used to generate reports of DCT data processing expenditures.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06402-06 ODCM

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Tumor Growth Kinetics and Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Stanley E. Shackney, M.D. Senior Investigator, OD, DCT, NCI

Other: Paul A. Bunn, M.D. Clinical Associate, Medicine Branch
Medical Oncology, DCT, NCI

COOPERATING UNITS (if any)

NCI-VA Oncology Section
Los Alamos Scientific Laboratory

LAB/BRANCH

Office of the Director, Division of Cancer Treatment

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

SUMMARY OF WORK (200 words or less - underline keywords)

In computer modeling studies the relationships among DNA content distributions, cell kinetics, and cell morphology were explored and applied in a proposed functional classification for the lymphomas.

In vitro studies of the effects of hydroxyurea and cytosine arabinoside were done in Sarcoma 180, exploring CxT effects on drug lethality, sublethal damage, and ^{3}HTdR uptake. Studies were completed on the effects of Colcemid on hematopoiesis in mice, with special reference to its stimulatory effects on thrombopoiesis.

Comparisons of methods of rapid radioautography were carried out. A commercial bacterial colony counter was adapted for semiautomated grain counting in smears, and cell counting in sections. Flow microfluorometry was used to perform multiparameter analysis of clinical samples. Although special emphasis has been placed on chronic lymphocytic leukemia, Sezary's syndrome, and the lymphomas, a broad spectrum of tumors was studied.

In vivo ^{3}HTdR studies were carried out in four patients with Sezary's syndrome. Percent labelled mitosis curves and labelling indices were obtained in normal skin and Sezary's cells, and the labelling patterns of circulating Sezary's cells and granulocytes were studied.

A treatment protocol for small cell carcimona was developed in collaboration with NCI-VA to explore applications of cell kinetics in clinical chemotherapy.

Project Description

Major Findings

1. Modelling Studies

The modelling studies of acute human leukemia and malignant melanoma were finally completed this year; these studies were submitted and accepted for publication in the Journal of Theoretical Biology.

Modelling of in vitro population growth and in vivo - in vitro transplantation has been carried out in anticipation of the accumulation of in vitro drug data from which in vivo drug responses might be predicted. To this end, the model was extended to include the simulation of DNA content distributions and their dynamic changes in response to drug perturbation. Results of these studies, presented at the 29th Annual Symposium on Fundamental Cancer Research in Houston this year, include the following:

i. DNA content distribution patterns appear to reflect distinctive patterns of DNA synthesis in human tumors, as exemplified by the lymphomas.

ii. These patterns may correlate with distinctive interphase labelling patterns, PLM curve features, and cell morphology.

iii. The correlations suggest a functional classification for the lymphomas that is relatd to their histopathology.

2. Experimental Studies

a. Drug studies in Sarcoma 180 in vitro.

Studies on the effects of hydroxyurea were extended over longer post-perturbation recovery periods, to see if sublethal damage is more pronounced after prolonged exposure to drug. Interestingly, it was found that there was no additional detectable damage accompanying prolonged drug exposure of up to 12 hours. Comparable studies on cytosine arabinoside in Sarcoma 180 in vitro are nearing completion. Differences that are discernable so far between cytosine arabinoside and hydroxyurea include the following:

i. DNA synthesis is depressed for longer periods of time in surviving cells following cytosine arabinoside.

ii. Prolonged drug exposure produces greater lethal and sublethal damage; unlike hydroxyurea both initial dosage and duration of drug exposure are important determinants in the drug response behavior of the population.

b. Experimental bone marrow studies

Dr. Bunn's studies on the effects of Colcemid on mouse bone marrow and peripheral blood elements were completed this year. It was found that the thrombopoietic effect of Colcemid could be divided into an early, direct, and specific stimulatory component, and a late, reactive, nonspecific component which coincided with the restoration of the myeloid and erythroid cells.

The sectioning of mouse marrows exposed to cytosine arabinoside before and after ^{3}HTdR at various dosages and intervals was carried out this year. We have begun processing of these sections to determine if there are regional marrow effects of a cytotoxic "phase-specific" agent, in the same way that there is regional ^{3}HTdR labelling.

c. Instrumentation

A commercially available bacterial colony counter was found by Mrs. Ford and Dr. Bunn to be suitable for counting radioautographic grains. Studies to validate the instrument for grain counting showed that instrument counts were as accurate as, and more reproducible than visual grain counts in smears. The instrument was also found suitable for counting cells at low power in tissue sections. This will prove to be useful in providing rapid measurements of marrow cellularity following cytotoxic drug administration.

d. Radioautographic Methodology

A study was undertaken to compare conventional low ^{3}HTdR *in vitro* radioautograpny, high ^{3}HTdR dose *in vitro* radioautography, and the liquid scintillation fluid method reported by Salmon (which employs high dose ^{3}HTdR *in vitro*). A preliminary examination of preparations from the marrow of a patient with neuroblastoma (see below) suggest that the liquid scintillation method offers no advantage over high dose ^{3}HTdR conventional *in vitro* radioautography, and that both produce radioautographs more rapidly than low dose ^{3}HTdR conventional radioautography.

e. Flow Microfluorometry

The Los Alamos Cell Sorter was delivered in March of 1975. Technical problems which were encountered over the first 8-10 months were anticipated for the most part, judging from the early experience of the Pathology group at DCBD, NCI who took delivery on a similar instrument a year earlier. These initial problems have now been resolved, and the instrument is in routine operation. Work done on the instrument to date includes the following:

i. DNA content distribution studies have been performed on the effects of hydroxyurea and cytosine arabinoside on Sarcoma 180 *in vitro*.

ii. A human diploid reference standard consisting of purified T lymphocytes was developed for quantitative evaluation of clinical material. This standard produces a sharp G_1 peak with a coefficient of variation in the range of 3.5 - 4.5%.

iii. A variety of clinical samples have been subjected to multiparameter analysis over the course of the last few months (see 3c below).

3. Clinical Studies.

a. Sezary's Syndrome

A protocol for human in vivo studies employing ^{3}HTdR was submitted and approved by the Clinical Research Committee and the Medical Board. Three patients with Sezary's syndrome have been studied with in vivo pulse ^{3}HTdR injection, before and after leukapharesis, and one patient has been studied with a long term (20 day) ^{3}HTdR infusion. Sets of serial radioautographs of skin and blood have been dipped, stored, and developed for all patients; multithresholded labelling indices as a function of time have been obtained in skin and blood and multithresholded PLM curves in normal epidermal cells and Sezary's cells are being obtained in the three pulse dose pre-leukapheresis patients and should be completed by July, 1976.

b. Neuroblastoma

Serial FMF studies were carried out on the marrow of a patient with neuroblastoma before, during and after chemotherapy with vincristine, cytoxan and adriamycin. Accumulation of marrow cells in the G_2 region was evident at 24-48 hours. At later time points there was an accumulation of cells in the early and mid-S region which persisted through day 9. ^{3}HTdR studies are in progress to determine the labelling intensity characteristics of the cells which persist in early S region of the DNA content distribution.

c. The role of Colcemid as a platelet sparing agent in clinical cancer therapy.

A pilot protocol has been prepared to explore Colcemid's potential platelet sparing effects in thrombocytopenic patients and in patients receiving chemotherapy. This protocol has been submitted for approval by the NCI Clinical Research Committee. It is anticipated that patient accural will come both from the NCI-VA Medical Oncolocy Section in collaboration with Dr. P. Bunn, as well as from other clinical branches within DCT.

d. Clinical Protocol Development - Small Cell Carcinoma

Modelling studies in mouse L1210 leukemia treated with ara-C have suggested that the failure to cure animals with large body burdens of tumor cells was not due to the increased body burden of cells per se, but to differences in drug response attributable to changes in population kinetic behavior (Shackney, CCR 54:399, 1970). This is supported by recent studies in experimental tumor systems in several laboratories. This concept was applied to human small cell carcinoma of the lung, where it was hypothesized that the inability to maintain good initial responses to chemotherapy may be due to changes in population kinetics with therapy. In collaboration with the senior staff of the NCI-VA Oncology Group a treatment arm in protocol "small cell IV" was devised, employing sequential blocks of therapy designed to treat progressively more rapidly

growing tumor cells. Preliminary observations have indicated that rapid regrowth occurred in the third block of therapy consisting of intensive ara-C treatment, suggesting that:

i. Even complete responses in this disease do not imply extensive log kill; rather, in patients with CR the body burden of tumor cells frequently remains just below the threshold of clinical detection.

ii. Since the overall changes in tumor size that occur even in patients with CR were relatively small, this may also imply that kinetic changes in the tumor over this range of sizes may be small as well.

iii. For the present, at least, it would be prudent to construct sequential schedules which consist of multiple drugs each of which is of proven activity in advanced disease.

Appropriate modifications are being introduced into the sequential arm of protocol "small cell IV" in close collaboration with the senior staff of NCI-VA Oncology Group.

e. Diagnostic Flow Microfluormetry (FMF) Studies

Multiparameter analyses of a variety of human cell samples were examined by FMF methods. Frequency distributions of cells by size and DNA content revealed that cells with slight but definite aneuploidy could be separated clearly from normal diploid cells on the basis of cell size. Since aneuploidy is presumptive evidence for malignancy, the diagnostic applications of the FMF instrument appear to be most promising. Findings of special interest so far include the following:

i. A comparison of lymphocytes from 7 CLL patients with the normal T lymphocyte reference standard indicates that CLL cells are generally the same size or slightly smaller than T lymphocytes. CLL cells are generally diploid or hypodiploid. In one case there was a broad spread of DNA contents into the hypodiploid and hyperdiploid regions. The possibility is being considered that worsening of clinical status may be heralded by cytogenetic changes in CLL as in the case of CML. Of course, CLL is extremely difficult to study cytogenetically, since CLL cells do not transform well in vitro, and FMF may prove to be not only the most convenient, but possibly the only way to monitor some CLL patients cytogenetically.

ii. In contrast to CLL, Sezary's cells tend to be slightly larger than T lymphocytes, and slightly hyperdiploid. This is in agreement with cytogenetic studies in this disease.

iii. A comparison of findings in the pleural fluid of a patient with childhood Sarcoma with conventional cytologic diagnosis suggests that conventional cytology may be picking up the large, hyperchromatic malignant G_2 cells, and missing the smaller malignant (i.e., aneuploid) G_1 cells which may

be 10-100 times more numerous. This may be a more general phenomenon which we are actively exploring. With the recongition of aneuploid G_1 cell as malignant it may be possible to increase the sensitivity of detection of tumor cells by 1-2 logs.

iv. Pleural fluids, ascites fluids, and tissue suspensions have been examined in a variety of malignant conditions but the number of samples from each is as yet quite small. Ovarian carcinomas appear to exhibit a distinctively broad range of aneuploidy and cell size in its G_1 cells. In other tumors the possiblility of distinctive patterns must await the analysis of a large number of representative samples.

Proposed Course

1. Modelling Studies

Multiparameter modelling studies in Sarcoma 180 in vitro will be continued during the coming year, the primary objective being to fit growth curves, PLM curves, labelling indices and FMF data in the absence of perturbation, and to fit FMF, labelling index, and growth curve data following drug exposure to hydroxyurea and cytosine arabinoside. A major reprogramming effort will be required this year to make the model compatible with changes in the computer-graphics package introduced into the DEC-10 computer system. Depending on the level of effort required to complete this reprogramming, it may be possible to initiate an exploration of simulated therapeutic strategies in human tumors this coming year.

2. Experimental Studies

a. Drug studies in Sarcoma 180 in vitro.

Over the coming year surveys of changes in FMF patterns over time induced by adriamycin, vincristine, bleomycin, and nitrogen mustard will be carried out as functions of dosage and drug exposure duration. These surveys will:

i. provide a basis for interpreting such patterns in man, and

ii. help us to decide which drugs might be studied in greater detail by cloning and ^{3}HTdR methods, both singly and in combination schedules.

b. Bone Marrow Studies

Regional effects of ara-C on mouse bone marrow will be studied in tissue section following both high and low dose drug exposure and ^{3}HTdR at intervals. The experiments have been done, and the tissue sections have been cut. Radioautographs will be prepared and processed this coming year.

c. Megakaryocyte Maturation

Dr. Paul Bunn has indicated that he would like to pursue collaborative FMF sorting studies on megakaryocyte maturation in mice during the coming year. The feasibility of such studies will depend on the degree of success achieved in preliminary megakaryocyte enrichment of marrow cell suspensions. These studies will define the effects of various drugs on megakaryocyte production and maturation.

d. Instrumentation

In accordance with an interagency agreement with ERDA, the Los Alamos Scientific Laboratory will be working with us to explore improvements in coulter measurement on the cell sorter, to improve fixation and staining methods for FMF analysis, and to extend software development for the Los Alamos Cell Sorter.

3. Clinical Studies

a. Sezary's Syndrome

It is anticipated that at least one additional patient with Sezary's syndrome will be studied with an ^{3}HTdR long term infusion during the coming year. The processing of radioautographs in this patient, and the counting of radioautographs in the two patients that will have been studied with long term infusions should conclude our ^{3}HTdR studies in Sezary's syndrome this coming year.

b. The Lymphomas

A collaborative relationship has been established with Dr. T. Lincoln's group to perform integrated studies on the lymphomas. Morphologic and immunologic studies will be performed by them on samples which they will forward for multiparameter FMF analysis. It is estimated that we will be receiving 2-3 samples/week for analysis. The purpose of the study will be to establish a functional kinetic classification for the lymphomas, and integrate it with immunologic classification schemes.

c. Breast Cancer

A collaborative relationship has been established with Dr. R. Zucker of the Papanicolaou Institute, who has developed a method for obtaining single cell suspensions of human breast cancer. These suspensions will be fixed and sent to us for multiparameter analysis.

d. Miscellaneous FMF Analyses

It is anticipated that a variety of cell samples from patients with malignancy will continue to be received from the various clinical branches of DCT. Samples from normal patients (e.g., pleural effusions from patients with

pneumonia or congestive heart failure, and ascites fluid from patients with alcoholic cirrhosis) will also be required as negative controls. It is anticipated that many of these samples will be collected at the VA Hospital under the supervision of Dr. P. Bunn, who has a continuing interest in these studies.

It is hoped that during the coming year we will be able to streamline specimen analysis and interpretation and provide referring physicians with the information rapidly.

e. Other Studies

There is an interest in this laboratory in carrying out serial FMF studies combined with serial in vitro ^{3}HTdR studies in patients before, during, and after chemotherapy, in order to determine the nature and time course of drug damage in normal marrow and tumor when possible. There is also an interest in studying suitable patients with ^{3}HTdR in vivo, particularly those with lymphomas and sarcomas. It is anticipated that it will be possible to carry out many of these studies within the NCI-VA oncology program, and within other clinical branches of DCT, as dictated by the interest of senior staff.

Publications

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Shackney, S.E. The Role of Radioautographic Studies in Clinical Investigative Oncolocy and Chemotherapy. Cancer Treatment Reports. In press.

Shackney, S.E., Bunn, P.A., Jr., and Ford S.S. The Effects of Colcemid on Mouse Bone Marrow. Cell Tissue Kinet. In press.

Shackney, S.E. Interrelationships among the DNA Content Distribution, Cell Kinetics, and Cell Morphology: Theoretical Considerations, Experimental Correlations, and Clinical Implications. In Growth Kinetics and Biochemical Regulations of Normal and Malignant Cells. (Drewinko, B. and Humphrey, R.M., Eds.). In press.

Shackney, S.E. A Cytokinetic Model for Heterogeneous Mammalian Cell Populations. III. Tritiated Thymidine Studies: Correlations Among Multiple Kinetic Parameters in Human Tumors. J. Theoret. Biol. In press.

ITHSONIAN SCIENCE INFORMATION EXCHANGE
OJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL CANCER INSTITUTE
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07025-02 ODCM

ERIOD COVERED

uly 1, 1975 to June 30, 1976

TLE OF PROJECT (60 characters or less)

iomedical Engineering for Intramural Research, DCT

MES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER OFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Arthur D. Little, Inc. A. Wechsler, A. Burg, E. Drazen, J. Ketteringham, S. Battista, L. Peutner (Program/projects); C. Kensler, T. Heuchling, J. Kasten, E. Shanley (Mgt. Review); P. Thayer, I. Wodinsky, I. Miller, L. Young (MIT) (Prog. Consultants); D. Linsay, R. Liss, K. Wiig, J. Porter, J. Stevens (Projects)

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ffice of the Director, Division of Cancer Treatment

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NSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
14.0	13.0 (12 contract)	1.0

UMMARY (200 words or less - unclassified paragraph)

ustained collaborative biomedical engineering support, mainly of an exploratory/ evelopmental nature, has addressed the feasibility of engineering concepts and ethods; provided novel instrument research and development; and furnished nalytical support and technical consultation for clinical and pre-clinical nvestigations of the DCT Program during this period.

road engineering resources are being focused upon the characterization and mplementation of useful engineering projects for a diversity of intramural fforts in: tumor cell kinetics, whole body and regional hyperthermia studies, rug bioassay and drug screening, cell kinetic modeling, specialized clinical nstrumentation and products, tumor cell biology, and leukocyte procurement.

Project Description

General Objectives/Scope

1. To provide specialized and broadly based biomedical engineering (BME) resources for the Division of Cancer Treatment, NCI.

2. To identify and characterize problems amenable to BME solutions and to conduct BME research and development efforts responsive to the diverse research programs, projects and tasks of individual investigators of the Division of Cancer Treatment.

3. To develop and refine approaches for the effective management of broad BME resources through the contract mechanism.

Instrumentation - Tumor Cell Kinetics

During the past two years, the Los Alamos Scientific Laboratories (LASL) has accomplished the engineering and delivery of a flow cell microfluorimeter (FMF) system for tumor cell characterization and drug analysis studies by DCT (Shackney, Bono, et al). The development and installation of an extensive PDP-11/40 data collection and analysis program for four parameter cell sorting; the optimization of staining and fixation techniques for patient cell samples; and the provision of maintenance engineering represent principal LASL accomplishments during this period (Mullaney, Crissman, Salzman, LASL). Planned efforts emphasize new technical approaches for sizing small cells (potential sensing) and also extend preparative cell techniques and the characterization of selected tumor cell lines of interest to DCT.

Detailed plans for engineering of a TV based cell scanning system to complement FMF based measurements, were accomplished this period. This sytem will semi-automatically obtain DNA content measurements and radioautographic grain counts on the same cell, ultimately for the quantitative kinetic histopathologic correlations of tumor cells (Shackney, Bunn). This system evolved this period due to DCT staff activities that included; (a) discovery and adaptation of a low cost commercial bacterial colony counting instrument (Artek Systems Corp.) to accurately and rapidly count grains in autoradiographs and (b) the competative evaluation of a long standing instrument program for automatic grain counting and densitometry (Image Processing Unit, DCT).

Exploratory instrumental techniques that would obtain DNA content and synthesis rate information on a cell-by-cell basis or and with small populations of cells, particularly with flow cell technologies available, have been proposed by ADL projects. Some experimental progress has been made using multiple labelled isotopes and field emission spectrometry (Anbar, Stanford Research Institute). Refinement and expansion of new conceptual approaches are being considered, possibly for RFP advertisement.

Engineering improvements to the LASL cell microfluorimeter, which resulted in a data display package and cell sample flow probe, were accomplished by ADL during this period for cervical cell studies (Herman, DCBD).

Whole Body and Local Hyperthermia Projects

New activities have developed in support of whole body and regional hypertheramia studies planned by DCT (Bull, Atkinson, DeVita). Industrial proposals (Webb Assoc., Accurex Corp.) for whole body hyperthermia garments and control systems were received and evaluated. ADL examined possible areas for collaborative hyperthermia studies with DCT and submitted projects covering: the measurement of localized internal body temperatures by passive wireless implanted sensors; the effects of hyperthermia on adriamycin toxicity and therapeutic ratio (rat models); and techniques to increase the survival rate of rats heated to hyperthermia temperatures.

In support of anticipated regional hyperthermia/radiotherapy studies, as recommended by the DCT Board of Scientific Counselors, new contract efforts are being planned by the BME Unit to develop advanced equipment and techniques to control heat induction and to characterize thermal fields in both clinical and animal studies.

Drug Bioassay - Instrumentation

The feasibility of using bacteria with defective DNA reparase systems for the rapid bioassay of adriamycin, BCNU, and l-phenylalanine mustard was conducted (ADL subcontract) by using differential light scattering techniques (Differential III Model, Science-Spectrum, Inc.). The adriamycin assay was effective from 0.1 to 3 μg/ml with the general sensitivity range indicative of .03 - 1 μg/ml levels for other drugs tested. These data, combined with previous methotrexate sensitivity determinations (SRI) were used to survey clinical and research pharmacologists and clinical microbiologists associated with DCT intramural and contract programs (Burg, ADL). The evaluation indicated the instrument system could contribute to clinical pharmacology for multiple uses. However, present sensitivity and specificity will limit applications in the clinic. The precision of the instrument in research pharmacology, in contrast to competative analytical methods, seems to limit its acceptance. There appears to be an important need for this or similar instruments in clinical microbiological laboratories.

Drug detection methods proposed and partly evaluated this period are; (a) the application of high pressure liquid chromatography techniques (Ketteringham, ADL) for the detection of maytansine in body fluid at low concentrations (Adamson) and (b) the quantitation of anti-tumor drugs in body fluids by changes in the bioluminescence of photobacteria (NASA, Goddard).

Drug Screening - Pattern Analysis

A pattern analysis project (Entropy Limited, Carnegie Mellon University, ADL) to extract physical/chemical features of drug compounds showing correlation with anti-tumor activity was completed and reported this period to members of DRD Branch and to program planning groups of DCT. Although the results are introductory due to the limited size of sample drugs studied for L1210 activity, the study indicates there are sixteen structural features which could be potentially useful in the theoretical optimization of biological screens.

Mathematical Modeling - Cell Kinetics

During this period, engineering support (Wiig, ADL) was concentrated on the analysis of tumor-growth kinetics (Woo) of tumors both free-growing and subject to drug control. Ascites tumors were emphasized and solid tumor studies were started. Tumor growth in the spleen was also studied as a separate subsystem for the measurement of polyamines. A major portion of work was directed to complete a model framework for routine analytic investigations of new tumor systems and to include in the model the synthesis of biologic markers (polyamines) by tumors. Specific tumor/drug systems studied included L1210 and Burkitt's lymphomas with BCNU, ara-C and MTX. Initial work was performed using the kinetic model framework developed in previous years for optimal drug scheduling via control theory and computer simulation. Twelve working memoranda, three addenda to previous working memoranda, and two articles were published.

Collaborative studies (Woo, Wiig) have begun; (a) to perform quantitative analysis of multiple biologic markers (polyamines, nucleocides, CEA) in carcinomas of the breast and the colon/rectum to predict the disease course of patients, (b) to characterize the biologic marker content distribution by combining a cell age distribution (using DNA content as proxy) with the market content per cell in the cell cycle, and (c) to characterize the dynamics of tumor-drug interactions expressed by antitumor agent perturbation of cellular DNA content in individual cell cycle stages. The objective is to show therapeutic responses of antitumor drugs on DNA content distribution.

Clinical Instrumentation and Projects

This period, a collaborative engineering project (USNWC, NHLI, DCT) has completed a prototype device which should significantly increase the clinical quantitation of growth in colonies of marrow granulocyte precursors. This device, adapting a detection principle used in the Sidewinder missle system, will support studies of abnormalities in white cell proliferation and hematopoietic regulation mechanisms (Bull, et al). Preliminary analysis (Atkinson, et al) of instrument vs manual bone marrow colony counts shows a high correlation in accordance with theoretical predictions. Present evaluation indicates limited engineering refinements, primarily in rotating reticle pattern design. Industrial sources (Becton-Dickinson & Co., Artek Systems, Inc.) are being encouraged to build and market improved versions through technical documentation and consultation from the DCT staff.

Engineering approaches were developed and evaluated for a variety of clinical problems including: the acquisition of lymphocytes from cerebrospinal fluid without morphological damage (Poplack); the *in vivo* measurement of *intratumor* and tumor tissue interface metabolic rates and PO_2 using a percutaneously injectable O_2 sensor (Young, Atkinson); the use of percutaneously insertabile thermistor probes for early detection of mammary carcinoma (Sears).

Three improved bacteriological isolation garments (Stevens, ADL) for protective environment studies (Pizzo) were delivered this period. Variable and higher airflow permits better cooling for different activity levels. Improved reliability is expected from new battery units. Noise control is being evaluated.

Instrumentation - Tumor Cell Biology

A new project was established in collaboration with LTCB (Donlon, Sarin) to search for antibody or antigens of leukemic virus in human serum by automated affinity chromatography. A unique ORNL system will process small volumes of serum samples repetatively with short cycle times over a column of immobilized viral proteins (or protein antibody) for specific extraction of antibody. Absorbed antibody is eluted, dialized and concentrated for further assay. Initial objectives will determine the sensitivity and limitations of the procedure for expanded use with virus proteins or natural antibodies to these proteins.

A semi-automated chemical bench apparatus is being developed (Puesner, PBA/ADL) to support the extraction of reverse transcriptase from patient tumor material that is increasingly available and desirable to process by the LTCB (Saxinger). An extremely flexible system design of sensors, valves, programable electronic units, etc. will permit ready adaptation to meet new lab protocols and is expected to attract broader laboratory interest.

An investigation and report (Lindsay, ADL) into the limits of sensitivity and spatial resolution of current electron microprobe techniques for locating trace element markers in cells (Gillespie, Levine) was completed this period. Six advanced scanning transmission electron microscopy instruments were used to determine effects of parameters of electron-imaging and x-ray microanalysis of iodine-labeled nucleic acids. Experiments were performed on the ADL Stereoscan instrument. It was concluded that the experimentally derived MDL value of 3×10^{-17} gm for iodine, contained in an organic polystyrene matrix, was a tenfold improvement over results of ADL's previous work but was still short of the theoretical limit predicted by Russ of 3×10^{-18} gm. This sensitivity was considered of no current value in the detection and location of labeled nucleic acids within mammalian cells.

Electron microscopic characterization of Epstein-Barr herpes virus in cell culture (Liss, ADL) was continued this period in support of studies (Pizzo, Magrath) to improve the yield of virus from cells and establish a basis for biochemical studies of EB virus in human disease.

Fabrication of an electro-optical apparatus for electric dichroism and birefringence studies of macromolecules in solution (Levine, Glaubiger) was investigated and proposed this period (Lyddy, ADL).

A systemtic review of the literature and existing instrumentation for continuous flow electropheretic separation techniques, potentially useful in the processing of large numbers of virus tranformed cells was conducted this period (Penland, ADL staff) in collaboration with LTCB (Sarin). Limited experimental trials with advanced instrumentation (Beckman Instruments, Inc.) and reviews of NASA programs in bio-processing in space (Cell Separation Conference, Tuscon, Arizona, 3/76) have indicated no existing high resolution preparative technique of practical, short term value for LTCB.

Instrumentation - Leukocyte Procurement

Continued progress was demonstrated by the Oak Ridge National Laboratories (Breillatt, Remenyik) during this period with both axial and radial flow prototype rotor developments and in preclinical trials with dogs. Granulocyte recovery efficiencies have averaged approximately 70% for both designs over 16 axial and 6 radial experiments with human and dog blood. The radial flow concept was selected and is being fabriated for early clinical trials at DCT due to advantages of automatic control and production advantages inherent in disposable rotor parts and simplified seals. Theoretical models for blood sedimentation were extended to predict granulocyte efficiencies from bloods of different hematocrit and erythrocyte sedimentation rates under various operatconditions. Modifications to the automatic control system for the radial flow rotor were made and tested in DCT clinical trials.

A collaborative study (Graw, DCT/Liss, ADL) to define the effects of various processing modes and eluents (saline, ACD solution, etc.) on cell morphology of granulocytes from nylon fiber collection systems (Leukopak) was completed this period. Cytopathologic lesions in granulocytes populations were classified under a scoring system and cells collected by fiber and centrifugal processing were assessed for damage by transmission and scanning electron microscopy. Granulocytes most readily separated from the nylon fibers irrespective of the eluent, together with granulocytes from centrifugal separations, exhibited fewest morphologic lesions. Adherent cells were found extremely labile to iatrogenic dysfunction with the incidence of lesions correlating to the degree of cell manipulation used to promote separation from the nylon fibers.

Major Consultations

DCT investigators were provided consultation on the following subjects principally by ADL specialists; Definition, Planning and Evaluation of Individual Biomedical Engineering Projects for DCT (Penland); Measurement of the Microwave Absorbtion of Biological Material at Millimeter Wave Frequencies (Atkinson); Techniques for Locating Pulmonary Tumors via Radio-labelling, Ultrasound, and Temperature Measurement (Cohen); Techniques for Improving Catheter Couplings, Sterility, Reliability and Skin Fixation (Brennan); Removal of Bronchial Tumor Obstructions with Thermal Techniques (Cohen); Fabrication and Delivery of Quartz Centrifuge Sleeves (Gallagher); Review/Evaluation of Blood Cell Separator Development Program at ORNL (Penland, Graw); Concepts for Improved Tine Test Applicators (Rosenburg); Approaches to Illuminate Surgical Cavities (Rosenburg); Microscopic Characterization of Liposomes (Sarin).

Significance

This biomedical engineering activity relates to a significant number of problems within the Division of Cancer Treatment that can potentially benefit from bioengineering approaches. This activity is successfully focusing diverse technical resources to support DCT laboratory and clinical research. It is broadening the scope and data base of the biological and medical scientist to achieve specific research goals. It thereby increases the probability that new engineering methodologies and instrumentation will contribute as in the past, to short and long range goals of the Division of Cancer Treatment.

Proposed Course

Major emphasis will be given to identifying key technical problems of DCT research programs that are amenable to BME approaches. To do this, physical scientists and engineers with multidisciplinary backgrounds and interests, who are new to the program, will be encouraged to investigate and contribute to current instrumentation and methodologies used in NCI laboratories and clinics. Special consultant teams outside of the NCI may also be utilized. More attention will be given to particularly complex long term problems by technical assistance beyound present contract resources.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 09000-02 ODCM

OD COVERED

July 1, 1975 to March 31, 1976

TITLE OF PROJECT (80 characters or less)

Hemopoietic Stem Cells in Human Peripheral Blood

ES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER ESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI : R. D. Barr, Visiting Scientist, OADP, NCI

Other: S. Perry, Special Assistant to the Director, NIH
J. Whang-Peng, Senior Investigator, MO, NCI

COOPERATING UNITS (if any)

edicine Branch, Medical Oncology, NCI
aboratory of Tumor Cell Biology, NCI
Department of Anatomy, Johns Hopkins University

LAB/BRANCH

Office of the Associate Director for Program

SECTION

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INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.1	1.4

SUMMARY OF WORK (200 words or less - underline keywords)

Hemopoietic stem cell concentrates have been prepared from normal human peripheral blood. Current studies evaluating this material involve:

1. Attempts to clone the stem cells in vitro and in vivo;
2. Assessment of transplantability of these cells in vitro and from in vitro to in vivo cultures, as a measure of their potential for self-replication;
3. Investigation of cell-cell interaction involving stem cells and syngeneic thymus-derived lymphocytes;
4. Estimation of the role of steroid receptors in hemopoiesis; and
5. Labeling experiments with ^{51}Chromium, ^{3}H-Thymidine, ^{3}H-Uridine, and ^{3}H-Glucosamine to define the stem cell metabolism and progeny in culture.

Project Description:

Following the successful identification of the human hemopoietic stem cell, attempts have been made to further characterize this entity. Particular efforts have been made to assess its potential for self-replication by cloning such cells in vitro, harvesting the colonies and effecting further cloning. As an extension of these studies the successfully cloned material was transplanted to in vivo cultures in order to evaluate its pluripotentiality. The stem cell suspensions could be consistently recloned once but not serially, a feature which may, in large measure, reflect the technical limitations of the systems employed. In vivo transplants revealed no evidence of pluripotentiality, probably reflecting the induction of commitment to granulocyte-macrophage differentiation in the preliminary in vitro cultures.

^{3}H-TdR labeling studies have served to further evaluate the cell cycle status of the stem cell concentrates. The results corroborate earlier data which suggested that the majority of these cells were out of the proliferative cycle, in a G_0 phase. Successfully triggering the cells to enter the growth cycle has been achieved by stimulation with viable syngeneic thymus derived lymphocytes. This finding may have considerable physiological relevance. A link with the myelostimulatory effects of corticosteroids was explored by determining the nature of steroid receptors on human lymphocyte subpopulations. These data may afford a measure of explanation for the clinical efficacy of steroid hormone therapy.

As a means of simplifying the isolation procedures involved in preparing hemopoietic stem cell concentrates, in the hope of eventual clinical utilization, a cell filtration technique, involving ordinary cotton wool, has been extensively tested in the laboratory. It appears capable of producing satisfactory material which may prove to be a more acceptable alternative to bone marrow in restoring hemopoietic integrity in patients who have suffered iatrogenic hemopoietic suppression as an inevitable accompaniment to the aggressive successful systemic approaches to the therapy of cancer with curative intent.

In the search for additional sources of hemopoietic stem cells, attention has been directed to the spleen. Studies in mice suggest that this organ is potentially suitable for providing further material for supportive care.

The present project will terminate when the contract of the principal investigator expires on March 31, 1976.

Publications

1. Barr, R. D., Whang-Peng, J. and Perry, S.: Hemopoietic stem cells in human peripheral blood. Science 190:284-285, 1975.

2. Barr, R. D., Sarin, P., and Perry, S.: Terminal transferase in human bone marrow lymphocytes. Lancet 1:508-509, 1976.

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4. Barr, R. D., Dougherty, W., and Perry, S.: A reappraisal of cellular labeling with radiochromium salts. J. Lab. Clin. Med. (in press).

5. Barr, R. D. and Perry, S.: Oncogenesis in human acute leukemia. Blood Cells. (in press).

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7. Harrison, T. A., Barr, R. D., McCaffrey, R. P. Sarna, G., Silverstone, A. E., Perry, S., and Baltimore, D.: Terminal deoxynucleotidyl transferase in AKR leukemic cells and lack of relation of enzyme activity to cell cycle phase. Biochem. biophys. Res. Commum. (in press).

8. Hirshaut, Y., Reagan, R. I., Perry, S., DeVita, V., and Barile, M. F.: The Search for a Viral Agent in Hodgkin's Disease. Cancer 34: (4), pp. 1080,1089, 1974.

9. Perry, S.: The International Effort in predictive screening for anti-tumor drugs: Critical problems. In Description of Systems Used in Experimental Screening of Anti-Cancer Preparations in Sixteen Countries, World Health Organization, International Conference on Screening Methodology for Anti-Tumor Drugs, Geneva, 1974, pp. 3-5.

10. Fliedner, T. M., and Perry, S. (Eds.): Workshop on Prognostic Factors in human acute leukemia, Reisenburg, Germany, October 1-3, 1973, Advances in the Biosciences 14, G. Raspe and S. Bernhard (Eds.) Pergamon Press, Oxford, pp. 1-638, 1975.

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G. Raspe and S. Bernhard (Eds.), Pergamon Press, Oxford, pp. 321-328, 1975.

13. Rosen, P. J., Eyre, H. J., Lilien, D. L., and Perry, S.: Study of Leukocyte kinetics in acute myelocytic leukemia utilizing Chromium-51. Medical and Pediatric Oncology, 1:51-62, 1975.

14. Perry, S.: Concepts in Cancer Chemotherapy (in Japanese), Cancer and Chemotherapy: 2:159-166, May 1975.

15. Sarna, G., Omine, M., and Perry, S.: Cytokinetics of human acute leukemia before and after chemotherapy. Europ. J. Cancer, 11:483-492, 1975.

16. Lippman, M. E., Perry, S., and Thompson, E. B.: Glucocorticoid Binding proteins in myeloblasts of acute myelogenous leukemia. Amer. J. Med., 59:224-227, 1975.

17. Kim, M. and Perry, S.: System identification and optimization of cancer chemotherapy. Workshop at Finsen Laboratory, Finsen Institute, Copenhagen 1975, by N. R. Hartmann, Edit. by A. J. Valleron, In: Mathematical models in cell kinetics, pp. 79-80, 1975. Pub.: European Press Mdikon, Ghent, Belgium, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09050-01 ODCM

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Bone Marrow Granulocyte Precursor Colony Counter

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. R. Atkinson	Physical Science Project Officer	PSPO NCI
OTHER:	J. Bull	Senior Staff Fellow	MO NCI

COOPERATING UNITS (if any)

U. S. Naval Weapons Center DOD

LAB/BRANCH

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SECTION

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

SUMMARY OF WORK (200 words or less - underline keywords)

A spatial frequency selective device for counting bone marrow granulocyte precursor colonies in a three dimensional gel media was conceived, designed, fabricated and is currently undergoing test and evaluation.

Objective

To provide a means of monitoring the growth of granulocyte precursor colonies in a turbid semisolid medium innoculated with bone marrow cells.

Methods Employed

An optical technique for automatically selecting spatial frequencies associated with growing granulocyte precursor colonies was devised. The technique was reduced to practice. An engineering development of an instrument embodying this technique was undertaken and a prototype instrument was fabricated. This instrument is currently performing as predicted and is under test, evaluation and calibration.

Publications

U. S. Patent 628,706 "Frequency Domain Discrimination and Counting Technique" assigned to the U. S. Government granted to E. R. Atkinson.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09051-01 ODCM

PERIOD COVERED

October 14, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Hyperthermia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E. R. Atkinson Physical Science Project Officer PSPO NCI

COOPERATING UNITS (if any)

Bureau of Radiological Health/FDA
Southern Research Institute
RCA Research Laboratory
Mayo Research Foundation
Montefiore Hospital, New York, N.Y.
U. S. Army Natick Laboratories
Colorado State University

LAB/BRANCH

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SECTION

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.75	0.75

SUMMARY OF WORK (200 words or less - underline keywords)

Methods of production, measurement and control of whole body and local hyperthermia are investigated. The systemic and local effects of such hyperthermia are investigated both alone and in conjunction with surgery, chemotherapy and radiotherapy to devise new and optimal methods of cancer treatment.

Objectives

1. To determine the therapeutic efficacy of hyperthermia, alone and in conjunction with surgery, radiation, immuno- and chemotherapy, in the treatment of cancer.

2. To optimize therapeutic procedures, techniques and schedules for the production and regulation of hyperthermic states safely and effectively.

3. To elucidate the mechanisms of action of hyperthermia in cancer treatment so that optimization may proceed in an efficient, rational manner.

4. To define the sequelae of hyperthermia, alone and in interaction with other treatment modalities.

Methods Employed

1. Animal Studies of Whole Body Hyperthermia

These studies have been aimed at discovering reproducible means, which would lend themselves to large scale testing, for the induction, sustenance and recovery from whole body hyperthermia. Six inbred strains of mice, commonly used for mouse and human tumor transplantation, have been exhaustively investigated as have Sprague-Dawley rats and New Zealand White rabbits. These investigations have included bath immersion, ultrasound, microwaves (2.24 GHz and 915 MHz), hot air at varying humidity, extracorporeal circulation, the use of pharmacologic agents and radiofrequency induction heating. In all cases, the cardiovascular response of the animal has dominated the experimental conditions used to induce whole body hyperthermia and, within reasonable limits, determined resulting time-temperature profiles independently of the external physical means employed. These effects have been shown to lend themselves to a very simple and highly reproducible physical model.

Controlled temperature-humidity chambers have been fabricated and installed at two contractor's sites for the exploration of a variety of tumor-chemotherapy-hyperthermia interactions in tumor bearing mice and rabbits. The relation of hyperthermia to adriamycin induced cardiomyopathy is under investigation.

Simultaneous electrocardiographic monitoring has been performed. $Beta_1$ adrenergic blocking agents have been shown to very significantly increase WBH tolerance as has simultaneous microwave exposure with other WBH modalities. The effort has been directed towards duplication of conditions thought to be effective for human subjects

and/or shown, in vitro, to produce a favorable therapeutic ratio between normal and tumor cell lines.

In all instances, tolerated conditions have been found to be exceedingly sharply defined (less than 20% in exposure time and less than 0.2°C temperature range). The concept of LD_{50} has little meaning in these threshold situations.

Lethal exposure to hyperthermia has been demonstrated to result in disseminated intravascular coagulation, heart block and hepatic failure. Primate studies have been undertaken to explore these sequelae. An isoenzyme analysis of lactate dehydrogenases and transaminases is being conducted on Rhesus monkies in an effort to identify effects presaging organ failure. A careful study of hyperthermia induced changes in hemostatic factors in primates has also been undertaken.

Profiles of hyperthermia induction which will produce minimal organ damage and cardiovascular insult to primates are being sought.

2. A protocol has been established for the controlled production of whole body hyperthermia in normal human volunteer subjects at the U.S. Army Natick Laboratories. The tests to be performed upon these subjects will roughly parallel the other primate studies being conducted. In addition, an attempt will be made to determine the performance characteristics of a circulating liquid heated garment which has been proposed for the clinical production of hyperthermia. Specialized instrumentation is available at Natick which would permit safe evaluation of these garments and experimentation with normal human volunteers.

3. Small Animal Studies of Local Hyperthermia

The efficacy of localized tissue temperature elevation as a means of cancer treatment is under investigation. This study includes methods of localized tissue heating by means of radiofrequency induction, microwave absorption, ultrasound absorption and limb immersion. Special emphasis has been placed upon the degree of localization obtainable by the various methods and the role of central nervous system regulation in producing oscillatory peripheral responses to locally imposed thermoregulation. The accessibility of internal organs to localized heating is being explored together with the use of microvasoactive pharmacologic agents for enhanced localization. Carbonyl iron appears promising in conjunction with transcatheter introduction and induction heating.

In an effort to dissociate the effects of a primary thermal lesion from possibly more effective differential tumor susceptibility to

elevated temperatures, an investigation of systemic sequelae of local hyperthermia is underway. These involve an examination of the growth and morphology of untreated tumors implanted in the same animal bearing treated tumors. Animals with varying degrees of immunocompetance (athymic, radioimmunosuppressed, chemoimmunosuppressed, and normal, as well as animals presenting already disseminated disease) are used to differentiate humoral and cell mediated effects on distant tumor sites. Tumors are chosen which metastasize selectively via venous and lymphatic channels. It was determined that microwave induced local hyperthermia ($43° \pm 0.5°C$) applied within three days of SQ implant of 100 L1210 cells in the CDF, mouse, produced total regression of the tumor. The effect of this localized treatment upon the known lifespan of animals suffering from disseminated disease produced by simultaneous IP implant of low L1210 innocula is under investigation.

Duration of survival of animals deliberately inadequately treated by local hyperthermia and bearing tumors known to proliferate selectively via venous and lymphatic paths is under study.

4. Temperature Measurement

Since hyperthermia presents unique problems of internal tissue temperature measurement, a number of techniques for the measurement of internal temperatures and gradients are under investigation. These include (1) ultrasonic imaging in phase rather than arrival time or amplitude, (2) passive microwave radiometry, (3) implantable transponders to microwave interrogation, (4) curie point transition ferroelectrics responding to microwave or optical interrogation.

5. Display/Alarm Systems

Since current studies in animals indicate that variation in exposure times measured in minutes and variation in temperatures on the order of 0.2 degrees centigrade constitute the difference between effective, ineffective and mortal results, an examination of state of the art methods of displaying and recording temperatures was undertaken. Platinum resistance thermometry is employed since this represents the best combination of inherent reliability, freedom from electrical interference and high stability. A system employing multi-modal alarms, displays and interlocks is being fabricated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 09052-01 ODCM

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Non-Thermal Effects of Microwaves on Living Tissue

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E. R. Atkinson Physical Science Project Officer PSPO NCI

COOPERATING UNITS (if any)

LAB/BRANCH

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SECTION

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INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

SUMMARY OF WORK (200 words or less - underline keywords)

The National Cancer Institute has solicited and is examining research projects dealing with the non-thermal effects of microwaves on living tissues as a possible approach to cancer treatment.

Objectives

This research knowledge is sought to assist in the exploration of non-traditional modalities of cancer treatment. Specific goals which may be supported are:

(1) the development of sensitive means to discriminate and identify tumor cells in normal host tissues,

(2) the discovery of differential tumor/host effects to be exploited alone or as a means of potentiating other modes of treatment,

(3) to further understanding of the basic mechanisms of tumorgenesis, and

(4) to provide a data base of organized quantitative parameters of malignancy to facilitate subsequent investigations.

Methods Employed

Microwave absorption spectra are to be measured on one or more of the following classes of tissues:

(1) representative normal and malignant tissues,

(2) normal and transformed cell lines,

(3) a variety of standard tumor strains in tissue culture where comparable normal strains are available.

These spectra will be determined with sufficient resolution and over sufficiently wide frequency intervals to permit correlation with previously established normal and tumor absorption progressions. Experimental conditions must be such that no appreciable sample temperature elevation occurs. Examination of measured samples by standard cytological techniques will be performed to the degree necessary to confirm identity and integrity. Thorough consideration should be given to the perfusion, nutrition, and mitotic state of tissues during measurements.

Examination of the influence of microwave absorption upon vibrational and rotational modes is to be performed by simultaneous observation of these modes by other physical probes.

Tentative attribution of observed microscopic effects will be made to possible events on an organizational, organelle, molecular, or transport level. This study of mechanisms of interaction is to be made in an effort to present observed effects in a meaningful context and to

provide guidance in seeking correlative observations. Consideration may be given to measurement of progressive spectral changes accompanying tissue treatment with known carcinogens or anti-neoplastic agents.

PUBLICATIONS SECTION

Office of the Director

The Publications Section directs the preparation of Cancer Treatment Reports (CTR) (the principal scientific journal developed under the DCT Program) and provides editorial assistance to the DCT staff on a time-available basis.

CTR (formerly Cancer Chemotherapy Reports) is now in its 18th year of continuous publication. From 1959 to 1968 CTR was issued 6-10 times a year depending on the acceptance of manuscripts submitted. Several types of manuscripts were published including program information, study protocols, experimental studies, and clinical reports. Most, but not all, manuscripts were reviewed by outside referees as well as the editorial board. In 1968 the journal expanded to three parts: Part 1 (reviewed manuscripts on original research, both experimental and clinical), Part 2 (comprehensive, lengthy chemotherapy studies involving a great deal of tabular material; some of the material was reviewed by referees), and Part 3 (program information including study protocols, clinical brochures, toxicology reports, and review articles; this material was normally not reviewed). In January 1976, CTR dropped the [illegible] system and began publishing monthly. In addition, the journal will now review manuscripts related to all areas of cancer treatment: radiation, surgery, immunotherapy, chemotherapy, and combined modalities. All original research material is reviewed. Every other issue now contains the proceedings of a symposium or seminar. The following outlines the activity of the three parts of CTR in 1975, prior to the institution of monthly publication.

Part 1: 1975.--In 1975 six bimonthly issues were published (total of 1206 pages; 179 manuscripts). A total of 215 research manuscripts were submitted to Part 1 in 1975. In addition, 55 manuscripts were accepted as part of symposia to be published.

Part 2: 1975.--In 1975 one issue was published containing the results of a screening symposium (19 manuscripts; 263 pages).

Part 3: 1975.--Two issues were published (54 manuscripts; 419 pages). One issue contained the proceedings of a high-dose methotrexate symposium; the other contained the proceedings of two seminars devoted to adriamycin, as well as a comparative clinical review of chemotherapeutic agents in solid tumors.

Submissions in 1976

In the first quarter of 1976 the journal had received the same number of manuscripts received in the first 2 quarters of 1975. Between April 1975 and April 1976 we received 10 requests to publish the proceedings of meetings. Although these increases will demand more editorial time, we are more frequently requesting that authors significantly reduce their manuscripts in length, thus saving time in editorial processing.

Comparative data on the CTR series of publications are shown in table 1. The manuscripts accepted are reported monthly to the Editorial Office of the Journal of the National Cancer Institute, which in turn distributes

this information in their monthly reports.

Sections of the Journal

Brief Reports.--In 1973, a section entitled Brief Reports and Preliminary Communications was added to the journal. Authors were invited to submit short clinical notes or the abbreviated results of preliminary work. These reports have been processed through the editorial channels more quickly than full-length manuscripts, thus reducing costs for production time and allowing more such manuscripts to reach the readers. The response to this new section has continued to be excellent during the past year. Each issue has contained 5-10 Brief Reports and an increasing number are being submitted.

Commentaries.--In 1974, a section containing invited editorials and commentaries was initiated; in the past 2 years, many such manuscripts have been received and published. A well-known scientist or physician is invited by a member of the Editorial Board to write briefly on a subject of current interest in cancer chemotherapy. We hope this section will encourage more interest in current topics, particularly through the submission of Letters to the Editor. In the past year we have accepted commentaries on the following topics: "Synergistic Interaction of Anticancer Agents: A Cellular Perspective"; "Current Status of Clinical Immunotherapy"; "Cancer Chemotherapy Agents and Carcinogenesis"; and "Laminar Air Flow Room Reverse Isolation and Microbial Suppression to Prevent Infection in Patients With Cancer." One guest editorial ("Combination Chemotherapy for Breast Cancer") was published in 1975.

Letters to the Editor.--During the past year CTR has observed a significant increase in the number of Letters submitted. These Letters usually, but not always, refer to previously published articles. Between May 1975 and April 1976 we have published (or have scheduled for publication) 20 Letters.

Clinical Notes.--The editors have briefly discussed the creation of a Clinical Notes section which will contain reports smaller than the Brief Reports and can be used to quickly bring to the attention of our readers new developments in cancer treatment. These notes will probably be similar to abstracts; readers can then contact the authors for more detailed information.

Proceedings of Symposia

During the past several years, CTR has been approached frequently to publish the proceedings of scientific meetings. In 1975, the proceedings of 7 symposia were published, and for 1976 we have published (or will publish) the proceedings of the following 7 meetings:

1. Sixth New Drug Seminar: DTIC
2. Metabolism and Mechanism of Action of Cyclophosphamide
3. Seventh New Drug Seminar: Nitrosoureas
4. Plants and Cancer
5. Tamoxifen Workshop
6. USSR Lung Cancer Meeting
7. Cell Kinetics and Cancer Chemotherapy

We have so far scheduled to publish the proceedings of the following meetings during 1977:

1. Workshop on Prostatic Cancer
2. Workshop on Antimetabolites and the Central Nervous System
3. Symposium on the Non-Hodgkin's Lymphomas
4. DEB Contractors Meeting

Cumulative Index

In 1974 the editorial staff began compiling a 9-year subject and author cumulative index for CCR Part 1 (1966-1974). Because of the extensive work required, the Publications Section contracted the completion of this work to a biologic abstractor with extensive experience in cancer research. The index has now been completed and is awaiting editorial processing. It will be published in late 1976. This year we will begin a cumulative index for all material published between 1968 and 1975 in Parts 2 and 3.

Official Charter for CTR

In April 1975, the first charter meeting of the CTR Editorial Board was held. This meeting was intended to resolve several issues concerning the organization of CTR, the name change to reflect the new direction of the DCT, and the additional duties of the Publications Section. In addition, the Board has written a substantive charter outlining such technicalities as the term of office for editors and the editor-in-chief (3 and 4 years respectively), the official policy regarding review and rejection, and the specific areas of research to be covered.

Editorial Board

In 1975 the Editorial Board expanded to include nine associate editors in addition to the editor-in-chief. Each year three editors will rotate off the board and three new members will be added. Provisions have been made to allow an additional year of editorship at the discretion of the editor-in-chief and the Director, DCT. In 1976, an Editorial Advisory Board with 15 members was established. The members will serve in a peripheral way to supplement the areas of expertise represented by the associate editors. Their advice regarding policy decisions, acceptance of symposia, and new areas to be covered will be solicited.

Coverage

Since 1967 CTR has been listed in Current Contents, Life Sciences. In 1973, CTR was asked to be included in a new publication of current titles in collaboration with Science, Engineering, Medical and Business Data Ltd., Oxford, England, and in 1974, we were asked to establish a similar policy with the Japan Medical Service, which publishes a supplement to its Index of Japanese Medical Periodicals listing foreign publications. It is expected that this additional coverage of the material presented in the journal will increase the demand for subscriptions, particularly in other countries.

In 1964, CTR began sending corrected page proof to the Chemical Abstracts Service for abstracting and indexing of the chemical information. During 1973 CTR was asked to establish a similar policy with the Biosciences Information Service (Biosis) in Philadelphia, and with Infordata International in Chicago, which publishes the Index to the Periodicals of the US Government. This trend toward more extensive coverage of CTR indicates a growing interest in the field and in CTR in particular.

Office of Management and Budget Approval

In late 1973 we submitted to the OMB our request for continuation of funds. This document outlined the purpose and scope of CTR, the publication costs incurred by the staff, a breakdown of the distribution of each issue (including the categories of subscribers), and the justification for continuing the publication. In early 1974 we received OMB approval which allows us to continue production through November 1976, at which time we will be required to request further funding.

Distribution of CTR

The Publications Section is responsible for maintaining the free mailing list for CTR. CTR is available without cost to qualified medical groups, physicians, and libraries, and is for sale to others by the Superintendent of Documents, Government Printing Office. The approximate distribution, which varies with each issue, is as follows:

3300	Foreign and domestic free mailing
1300	Superintendent of Documents (for paid subscriptions)
367	Superintendent of Documents (for Depository Libraries)
4967	

In the past several years, the number of paid subscriptions has increased tremendously. The number will undoubtedly continue to rise because of the lower limits set by the OMB each time the journal receives OMB clearance. The demand for specific back issues remains high; most requests are for the proceedings of meetings.

Problems with the service rendered by the GPO in maintaining the mailing list have brought about a change in the distribution procedures. Beginning in 1973 the mailing list was transferred to the Distribution Section, Printing and Reproduction Branch, ODA, NIH. This conversion allowed more rapid service in 2 areas: (a) adding, deleting, and changing names on the list, and (b) obtaining mailing tapes to be used by the contract printer in mailing the issues to the subscribers. NIH has been able to perform these functions in one fourth of the time GPO previously required. Despite these improvements in service, the Publications Section has been anxious to cut down the number of errors and the delay in adding or deleting names on the list. We have obtained a computer terminal which will allow us to process our own mailing labels. This will make it possible for us to add, delete, and change names within seconds. In 1975 we screened the mailing list to eliminate addresses of subscribers who have moved, retired, or died. This clearing of the mailing list allowed us to add names of many individuals that were on a waiting list.

Contract Manuscripts Clearance

For 8 years the Publications Section has been responsible for the clearance of DCT contract manuscripts submitted for publication in scientific journals. In 1975, 218 of these manuscripts were cleared and during the first quarter of 1976, 51 manuscripts were cleared. In assuming the responsibility for clearance of these manuscripts, the Publications Section checks all NSC numbers mentioned in the papers and notifies the DDB when compounds must have publication clearance from suppliers. The authors are instructed to reword footnotes as necessary to give proper credit to the DCT. The Section ensures that the Project Officer sees all papers and signs the correct forms. Records and reprints are kept for all manuscripts cleared, and quarterly reports are prepared for distribution to staff personnel.

In 1976 it was decided to drop this function of the Section for several reasons: only a small fraction of all DCT contract-supported manuscripts were submitted for clearance. Others were merely submitted to the appropriate journal and published without "official" approval by our section or the project officer. Due to the increasing workload of the section it seemed acceptable to request that project officers monitor their contractors with respect to the requirement of giving proper credit to the NCI for funds and deleting any reference to discrete compounds. Also, there exist differences between the several types of research contracts regarding the degree of project officer clearance required. Thus, it would be more efficient to let each project officer monitor his own contractors, since he or she would be more familiar with the contract specifications.

Table 1

Year	Part	Mss rec'd	Mss accepted	Issues published	Pages published	Reviewers	Total costs ($)*	Cost per page ($)
1971	1	107	73	5	621	164	33,284	54
	2	6	5	1	246		14,828	
	3	11	11	1	104		4,564	
		124	89	7	971		52,676	
1972	1	122	76	6	814	197	37,715	52
	2	4	5	1	353		21,064	
	3	47	47	2	181		10,863	
		173	128	9	1348		69,642	
1973	1	148	112	4	524	167	23,713	54
	2	19	19					
	3	77	76	3	576		26,184	
		244	207	7	1100		59,897	
1974	1	244	138	6	978	212	42,706	40
	2	21	21	4	758		25,069	
	3	21	21	1	87		4,550	
		286	180	11	1823		72,325	
1975	1	215	114	6	1206	294	66,000 (approx)	54 approx
	2	19	19	1	263		15,000 "	
	3	131	138	2	419		20,000 "	
		365	261	9	1888		101,000 "	
**1976 Research mss		113	59	12 planned	1850 approx		Not available	
Symposia		51	51					

*Includes the cost of printing, typesetting, and distribution only.
**From Jan 1 to May 10, 1976.

LITERATURE RESEARCH SECTION

Office of the Director

The Literature Research Section serves the Division of Cancer Treatment by providing information from published literature on all aspects of the therapy of cancer. Data from the fields of chemotherapy, radiotherapy, surgery, immunotherapy and the related chemical and biomedical disciplines are used by the staff in Decision Network review, meeting FDA requirements for IND filing, preparing clinical brochures, and as background for evaluation of toxicological and clinical studies.

Literature Services. The Section received and filled over 250 requests for information during the year. Responses were provided as comprehensive or selective bibliographies, computer print-outs, abstracts and copies of articles. More than half of the requests entailed manual literature searches supplemented by the various automated bibliographic retrieval systems such as Medline, Toxline and Cancerline. Extensive clinical searches were performed for treatment of cancer of the lung, cervix, and prostate and of the myeloid leukemias. Bibliographies were prepared, or updated, for such agents as daunomycin, the nitrosoureas, iphosphamide, sterigmatocystin, deazauridine, maytansine and hexamethylmelamine. Searches restricted in scope were done on aspects of treatment of various neoplasms and on the commercially available agents. A recurring bibliography on adverse effects of antitumor agents that NCI has in the clinic continues to be prepared.

The Section co-ordinates NCI access to the on-line data bases of the Medlars automated bibliographic search system. In this role the Section serves all areas of the Institute, processing searches and providing assistance and instruction in the use of the system. It is also the center for access to the Toxline and Cancerline data bases for personnel in the Blair Building. These computer services aid in the searching and facilitate the answering of requests. This year, responses to about fifty requests were in the form of computer print-outs only. Monthly SDI (Selective Dissemination of Information) bibliographies are produced for members of the staff on specific subjects of continuing interest.

The staff continues to be responsible for the preparation and distribution of the agent folders for Decision Network meetings. Each folder contains the structure and systematic name of the compound, a bibliography from the published literature, summaries of screening, toxicological or other preclinical or clinical data, and questionnaires. Materials were assembled for a total of 33 drugs during the year.

A further responsibility of the Section is the maintenance of the Cancer Therapy Library, a collection of books and journals for the use of NCI staff. Copies of over 60 journals are regularly received including many of the cancer journals, abstracting and indexing secondary sources, and chemical, biomedical or information science journals of special interest to the Section and other personnel in the building. During the year approximately $10,000 was spent on books and subscriptions.

Contract Operations. Contracts for the preparation of Cancer Chemotherapy Abstracts and its successor Cancer Therapy Abstracts have been monitored by members of the Literature Research Section since 1965. In addition to the printed journal and hard copy of all cited articles, computer tapes of the abstracts and indexes are received from the contractor. Under the sponsorship of the International Cancer Research Data Bank, these are input to the Cancerline file which can be searched on-line through the Medlars network.

SUMMARY REPORT

DRUG RESEARCH AND DEVELOPMENT PROGRAM (DR&DP)

DIVISION OF CANCER TREATMENT (DCT)

The Drug Research and Development Program has as its primary task the search for and development of new and potentially more effective antitumor agents, as well as the development of better methods of drug selection and prediction of optimal therapy with new and old drugs and drugs combined with other treatment modalities. With respect to the Division of Cancer Treatment (DCT) Linear Array, the organization is responsible for all preclinical work in the development of drugs except that concerned with toxicology and pharmacology.

The DR&D Program includes both in-house laboratory operations and contracts with pharmaceutical and chemical companies, research institutes and academic institutions, with the major portion of the funding directed toward the contract-supported activities. The program depends heavily on additional collaboration with research grantees, other independent investigators throughout the world, and hundreds of industrial companies not involved in contracts. The program activities cover the following specific areas:

1. Design and synthesis of new chemical agents.
2. Development of new agents from fermentation, plant and other natural products.
3. Procurement and resynthesis of bulk chemicals and drugs for preclinical and clinical evaluation.
4. Development and production of clinical dose forms for clinical trial.
5. Quality control for bulk and formulated drugs.
6. Procurement of radiolabeled drugs for clinical pharmacologic and experimental studies.
7. Primary screening and evaluation of new materials.
8. Bioassays, both in vivo and in vitro, to monitor the purification of fermentation and other natural products.
9. Study of optimal drug routes and regimens, including therapy with combinations of drugs and combined modalities.
10. Development of new and improved laboratory methods for discovering, evaluating and utilizing potential antitumor agents.
11. Investigation of mechanisms of action and resistance of drugs of clinical interest.
12. Investigation of new approaches to combined modality therapies, involving surgery, radiotherapy and immunotherapy in addition to chemotherapy.
13. Investigation of selected problems related to the biochemistry, cell kinetics and pharmacology of drug action.
14. Animal procurement, distribution and disease control.
15. Development and operation of data processing systems for both biological and chemical information.

TESTING OF NEW MATERIALS

A major change is underway with regard to the testing of new materials. There has been concern for some time that the use of a single *in vivo* primary screen is too limited and undoubtedly has missed materials that would be active in man. The practical problems related to screening large numbers of materials in many screens have precluded the establishment of what might be an optimal spectrum of systems for *de novo* screening. We believe that a compromise approach is feasible, however, and worthy of exploration.

Beginning in 1976, DCT intends to place greater emphasis on the testing of new synthetic compounds and natural product isolates against a spectrum of animal models of specific human tumors. Although the selection of all systems has not yet been accomplished, it is expected that this panel of animal screens (Figure 1) will include mouse colon, breast, and lung tumors. Human tumor xenografts in athymic mice will be included as the supply of these immunologically incompetent animals and testing capacity permit. Other animal tumors of specific interest, e.g., transplantable brain tumors, will be utilized in specific instances. The level of effort required to test a material in most of the systems in the spectrum is greater than the present effort for primary screening against transplantable mouse leukemias. This and the number of test systems involved means that the input to this panel of antitumor screens will be limited in number. This raises the obvious question of the basis for selecting materials for such broad spectrum screening.

After considering a number of alternatives, we have determined that the most reasonable approach would be to utilize, as an initial *in vivo* screen or "pre-screen", one which has demonstrated sensitivity to most classes of clinically effective anticancer drugs, but which, nevertheless, is sufficiently discriminating to avoid "overloading" the spectrum. The current screen for synthetic agents, mouse leukemia L1210, while known to be highly predictive for clinical utility against human leukemias, lymphomas, and solid tumors, was considered to be too restrictive for a "pre-screen" designed to minimize the chance of missing a compound likely to show activity in one of the tumors in the spectrum. A comprehensive review of existing screening data indicated that mouse leukemia P388 would represent a more appropriate "pre-screen" because (a) its response to drugs of various classes is qualitatively similar to that of L1210, and (b) it is quantitatively more sensitive than L1210.

The object of the "pre-screen", which must be limited to one *in vivo* system because of cost considerations, is to select a population of materials which will yield a higher percentage of "actives" in one or more of the spectrum tumors than would *de novo* screening in the entire spectrum. In addition, it is expected that the "pre-screen" will include an *in vitro* assay which will permit the selection, for subsequent *in vivo* spectrum screening, of highly cytotoxic materials and materials which may induce cellular differentiation, a potentially useful drug action which cannot be discerned using the present *in vivo* models or the KB cell culture screen. At this time we are investigating the Friend virus-induced erythroleukemia *in vitro* for its utility for this purpose.

Crude natural products will continue to be tested against KB cells in culture and against P388 in mice. The KB cell culture screen has been an efficient tool for bioassay of natural product fractions that show activity both *in vivo* and *in vitro*, and there is no reason to alter the current practice for these materials. Active isolates from the natural product screen will be tested against the tumor spectrum.

Despite the necessity of limiting our initial *in vivo* screen to one assay, we recognize that no animal model is known to predict perfectly for clinical utility. Therefore, we plan to augment the input to the tumor spectrum with materials with antitumor activity reported in the world's literature and from other screening programs, and with compounds reported to exhibit pertinent biochemical and other biological activities. Such a compound will be tested in the "pre-screens" to provide data for future analyses, but will be tested in the tumor spectrum regardless of pre-screening results. In a sense, then, agents which have shown interesting activity outside of our program will essentially "by-pass" the "pre-screen" as a barrier to broad spectrum screening.

Because of their demonstrated past utility, leukemia L1210 and B16 melanoma will be retained as part of the screening spectrum. Lewis lung carcinoma is still being used but alternative mouse lung tumor systems are being investigated. Congeners of known active drugs will continue to be tested in the specialized assays designed for each structural class.

As materials emerging as active from any of the spectrum models (or even very active in the P388 screen) are subjected to adequate clinical trial, this approach to screening will provide, prospectively, critical information relative to the value of specific animal tumors or combinations of animal tumors.

Active efforts have already been underway for several months to implement the changes outlined above. As indicated in Table 1, the input of new synthetic compounds to the program has already been cut substantially, from 39,814 in calendar year 1974 to 21,295 in calendar year 1975. It may take another year or more to decrease the input level to that projected for the future, since a significant backlog exists in the testing of new materials and some of the new experimental systems may take a considerable period of time to become operational. The backlog has resulted from a decrease in testing capacity (Table 2) and the need for more special studies.

It is worth discussing briefly the relative effort devoted to the screening of new synthetic compounds. Although the total effort in contracts under the auspices of the Drug Evaluation Branch amounts to approximately 10 million dollars, the primary and secondary screening of new synthetic compounds *in vivo* represents about 2.9 million dollars (plus the cost of animals). Thus, very substantial efforts are devoted to other aspects of drug evaluation such as the detailed study of active agents, combination therapy, development of new methods, etc. Any changes in the initial screening of new synthetic compounds would impact on only about 30% of the total effort in that area. A more detailed discussion of these factors may be found in the report of the Drug Evaluation Branch.

SELECTION OF COMPOUNDS FOR TESTING

It will undoubtedly be necessary to make some intellectual preselection of compounds for testing in our new experimental screening systems because of limitations in test capacity. In addition, however, we believe the time is arriving when a more "rational" approach to the selection of compounds for screening may be emerging. Extensive discussions have been held among NCI staff and with a number of outside consultants concerning the approaches one might take to the preselection of compounds for screening. A major factor will, of course, be the surveillance of published literature. Criteria are currently being developed to guide the NCI in selecting materials from the literature and in guiding such materials into the appropriate experimental systems based on the type of activity reported and the nature of the material. It is obvious that activity in an in vivo experimental tumor system in another laboratory would justify testing a new material in the broad spectrum of experimental systems that will be established. Other types of biological activities are not necessarily so clear in their significance, however, and further discussions may be necessary to establish priorities. There is the additional priority problem relating to materials to be tested in the pre-screen, however, since it is possible that more materials may be offered for testing than the capacity will allow. In these cases biological activity will also play a role, but once such rationales have been exhausted one must ultimately resort to selections based on structural characteristics. Decisions in this area are more difficult, but further discussions are being held in order to arrive at approaches that may be worthwhile. In the past a very active solicitation program was carried out to procure the rather large numbers of new synthetic compounds that were needed. The simple elimination of that acquisition effort will undoubtedly result in a substantial decrease in the input. However, that approach would not necessarily provide the most interesting compounds with the best probability of being active in our screen. Therefore, it would seem that a more deliberate attempt to select compounds should be attempted, although the specific methods cannot be specified at this time.

DEVELOPMENT AND STUDY OF NEW TUMOR MODELS

An increasing effort is now being devoted to the development and evaluation of a variety of new tumor models that might be used either for the initial screening of materials or for their further evaluation. As stated earlier, some of these models will be used as part of the total panel for secondary screening of compounds (Figure 1), and most of the effort is indeed going in that direction. Some of these models are already in active use such as the mouse breast carcinoma model at the Catholic Medical Center. Others, such as the human tumor xenografts, must still be developed, evaluated and placed into operational use. The specific tumors that may be used in those cases have not yet been selected. In the future there may also be additional emphasis placed on the use of a spectrum of tumors for the evaluation of analogs. In the past much of the effort has been devoted to seeking analogs with quantitatively superior activity in the same tumor system as the parent compound. Seldom is such superiority observed, however, and undoubtedly potentially useful compounds have been overlooked through this mechanism.

Therefore, some studies will be carried out to determine if a tumor spectrum would provide information of value in the selection of such analogs.

CORRELATION OF ANIMAL AND HUMAN TUMOR ACTIVITY

During the past year a Drug Evaluation Branch contractor, Arthur D. Little, Inc., in collaboration with Drug Research and Development Program staff and Cancer Therapy Evaluation Program staff has attempted to adapt quantitative statistical methodologies to both experimental screening and clinical data. They are developing formulas which might help determine which animal model or combination of models might predict best for individual human tumor types. Although the data are quite preliminary at this stage, the analyses carried out so far are quite encouraging and are actively being pursued further. A more detailed discussion of this topic is contained in the Summary Report of the Drug Evaluation Branch.

PROGRAM OUTPUT OF NEW DRUGS

As a result of the screening and related drug development efforts, eighteen materials passed Decision Network 2A in calendar year 1975. That is, they demonstrated sufficient biological activity and chemical or other uniqueness to warrant the effort in dose formulation development, etc., leading to preclinical toxicology and clinical trial. These materials are listed in Table 3. The coordinated preclinical drug development work in calendar year 1975 resulted in four IND applications being filed, signifying the initiation of clinical trials. These materials are listed in Table 4.

ORGANIZATIONAL CHANGES

During the past year a major organizational change occurred with the establishment of the Laboratory of Medicinal Chemistry and Biology, resulting from the consolidation of the Drug Design and Chemistry Section from the Drug Development Branch and the Molecular Biology and Methods Development Section from the Drug Evaluation Branch with components of the Laboratory of Chemical Pharmacology, ET, DCT, and other components from the Division of Cancer Biology and Diagnosis. The objective in establishing this Laboratory is to foster a closer relationship between medicinal chemists involved in the design and synthesis of new agents and biologists, biochemists and pharmacologists involved in their evaluation and further study. By bringing these components together and encouraging their intellectual interchange it is hoped that a more effective team approach to the design of new drugs can be created. As we move toward more rational approaches to drug design, teams such as this should play an increasingly important role. Much of the effort within the Drug Design and Chemistry Section has been devoted to the development of agents for the treatment of central nervous system disease and has in fact resulted in the selection of one material for further development toward clinical trial during 1975 (NSC 172112, Table 3). The interrelationships created through the establishment of this Laboratory will undoubtedly foster more rapid developments in this as well as other areas of drug design and development.

Another major organizational change is in the process of being implemented. As noted in the report of the Drug Development Branch, the activities there fall into three major areas: (1) development of new synthetic compounds, (2) the development of natural products, and (3) applied services relating to large-scale production, formulation, etc. The plan being implemented will divide that activity into three branches. The first will be devoted to the development of new synthetic materials by means of contract synthesis and by acquisition of new synthetic materials from the scientific community. Included in this branch will be studies on structure-activity relationships and problems related to preselection and ancillary chemical information activities. A Natural Products Branch will incorporate the activities currently being carried out in the Natural Products Section of the Drug Development Branch, and will provide additional emphasis for that activity. The third branch will be devoted to the further development of materials discovered in either of the other two branches. Thus, it will be concerned with the large-scale production of bulk synthetic chemicals, the development of clinical dosage formulations for both oral and parenteral use, production or procurement of formulated drugs for clinical studies, preparation of radiolabeled drugs for clinical pharmacology and other studies, the distribution of clinical dosage forms to clinical investigators and the analytical and quality control activities necessary to control the bulk and formulated materials destined for use in man. Since this organizational change has not as yet been implemented the present annual report of the Drug Development Branch is presented as a single unit.

Figure 1

Proposed Flow of Drugs Through DCT Screens

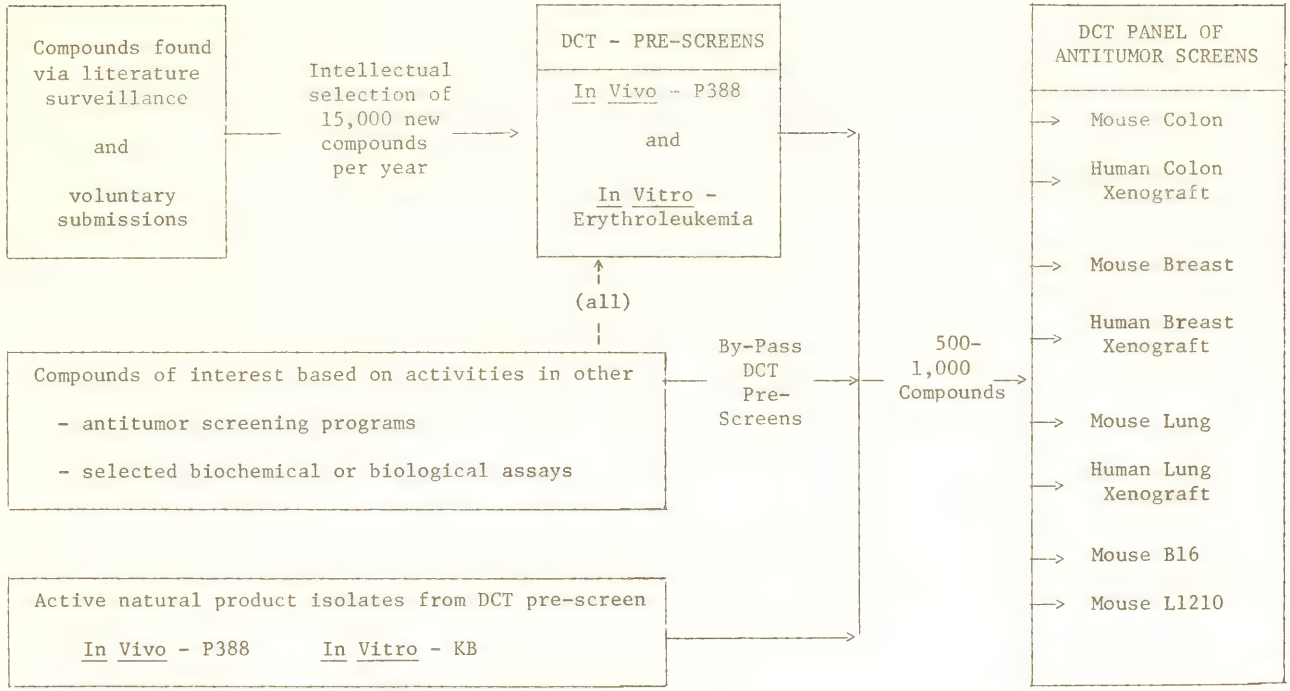

Table 1

Input and Output of

Drug Development Program

Materials Submitted	Calendar Year									
	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975
Synthetic & crystalline natural products	8,008	7,413	10,915	9,820	8,521	10,151	15,770	34,143	39,814	21,295
Crude fermentation products	4,284	4,982	4,256	2,184	2,172	1,215	2,712	2,550	725	1,328
Crude plant products	3,620	6,253	4,245	5,109	3,499	3,015	10,434	11,661	5,830	6,393
Animal products		446	641	757	587	1,203	1,669	2,689	2,011	2,086
Total	15,912	19,094	20,057	17,870	14,779	15,584	30,585	51,334	48,380	31,102
IND applications filed	3	4	5	5	6	8	5	13	3	4

Table 2

Screening Tests

	Calendar Year							
	1968	1969	1970	1971	1972	1973	1974	1975
L1210 Lymphoid Leukemia	107,445	80,545	79,892	102,424	103,681	133,481	210,862	159,118
P388 Lymphocytic Leukemia	14,903	43,223	42,270	53,449	180,027	198,711	123,964	125,652
Walker Carcinosarcoma 256	44,563	20,328	10,247	4,368	2,770	--	909	1,318
B16 Melanoma	286	1,240	1,342	1,423	6,028	15,989	11,724	8,837
Lewis Lung Carcinoma	244	925	3,033	2,803	4,141	2,441	2,770	5,847
AK Leukemia	--	--	--	--	--	--	925	462
Other Tumors	774	426	504	1,780	6,458	5,493	4,223	5,000
Total In Vivo	168,215	146,687	137,288	166,247	303,105	356,115	355,377	306,234

Table 3

Compounds Passing Decision Network 2A

NSC No.	Compound Name	Basis for Selection	Source
5159	Chartreusin, dihydrate	P388 and B16	Fermentation
59265	Hematoporphrin	Effect on photoradiated tumors	Animal
69587	Metronidazole	Radiosensitizer	Synthetic
95466	Urea, 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitroso	Mouse brain tumors, advantageous partition coefficient	Synthetic
137679	Guanosine, 6-seleno-	L1210, Mecca, $6C_3HED$	Synthetic
169780	ICRF-159 (+)-	Soluble form of ICRF 159	Synthetic
172112	Spirohydantoin Mustard	IC mouse ependymoblastoma	Synthetic
192965	2-Aza-8-germaspiro[4.5]decane-2-propanamine, 8,8-diethyl-N,N-dimethyl-1, dihydrochloride	Reported clinical activity	Synthetic
204985	Sterigmatocystin	P388 and L1210	Fermentation
208642	FILIPIN, a Pentaene	P388 and B16	Fermentation
224131	PALA	B16 and LL	Synthetic
227262	Compound from Bouvardia ternafolia	P388 and B16	Plant

Table 3 (Continued)

Compounds Passing Decision Network 2A

NSC No.	Compound Name	Basis for Selection	Source
246112	Hydrazinecarbothioamide, 2-[(5-amino-4-methyl-1-isoquinolinyl)methylene]-	L1210, biochemical and pharmacological superiority over parent	Synthetic
246131	AD-32	L1210, superior to adriamycin	Ferm/Synthetic
247561	Ledacrine	Clinical reports and W256	Synthetic
249992	Methanesulfonamide, N-[4-(9-acridinylamino)-3-methoxyphenyl]	Improved form of NSC 141549	Synthetic
261036	1,2-Propanediol,3-(2-nitro-1H-imidazol-1-yl)-	Radiosensitizer	Synthetic
261037	1H-Imidazole-1-ethanol, α-(methoxymethyl)-2-nitro-	Radiosensitizer	Synthetic

Table 4

IND'S Filed

NSC No.	Compound Name	Source
126771	1,4-Naphthoquinone, 2-(3,3-dichloroallyl)-3-hydroxy	Synthetic
141537	Anguidine	Fermentation
150399E	Spironolactone	Synthetic
153858	Maytansine	Plant

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

HEALTH, EDUCATION, & WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CM-03575-03 DR&DP

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (60 characters or less)
Chemotherapy and Combined Modality Investigations of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Abraham Goldin	Chief, Laboratory Research	DR&DP NCI
OTHER:	Ira Kline	Expert Consultant	LEC NCI
	Randall K. Johnson	Actg. Head, Tumor Biology Section	LEC NCI
	David P. Houchens	Senior Staff Fellow	LEC NCI
	John M. Venditti	Chief, Drug Evaluation Branch	DEB NCI
	Omar C. Yoder	Chemist	DR&DP NCI

COOPERATING UNITS (if any) G. Cudkowicz, State Univ. of N.Y.; J. Venditti and staff, H. Wood, Jr., R. Engle, J. Douros, D. Johns, J. Driscoll, J.A.R. Mead, S. Carter, F. Muggia, M. Slavik, M. Rosenzweig, NCI; H. Skipper, F. Schabel and staff, Southern Research Institute; I. Wodinsky, I. Miller, S. Stone, A. D. Little; E. Frei, Children's Cancer Res. Foundation; E. Bonmassar, Univ. of Perugia Med. School; L. DiMarco, Farmitalia; A. Nicolin, Univ. of Milan; S. Garattini and F. Spreafico, Istituto di Ricerche Farmacologiche "Mario Negri".

LAB/BRANCH
Laboratory of Experimental Chemotherapy

SECTION

INSTITUTE AND LOCATION
NIH, NCI, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to: a) plan and coordinate chemotherapy and combined modality research projects within the Drug Development Program, b) perform structure-activity studies on important classes of antineoplastic agents, c) explore new and potentially useful biological and biochemical methodology for evaluation of antineoplastic agents, d) evaluate and correlate preclinical activity of antitumor agents with clinical activity, and devise improved methods for the selection of drugs with potential activity in human cancer, e) conduct international activities for the acquisition and screening of new and potential antitumor agents abroad.

Project Description:

This project in the Office of the Associate Director, Drug Research and Development encompasses the following objectives:

I. To contribute to the planning and coordination of the chemotherapy and combined modality research program of Drug Research and Development and application of drug development and total program.

II. To conduct fundamental investigations on special problems of program interest.

III. To interdigitate with the Chemotherapy Laboratory, the Medicinal Chemistry and Biology Laboratory, Drug Evaluation and Drug Development programs of Drug Research and Development and other preclinical programs in the Division of Cancer Treatment, in structure-activity studies and in the exploration of new and potentially useful biological and biochemical methodology for evaluation and improvement of drug therapy and in special problems and new approaches.

IV. To collaborate with the clinical program, Division of Cancer Treatment in areas interrelating preclinical and clinical investigations. Increasing emphasis is being placed on the combined modalities of radiation plus chemotherapy including radiosensitizers and radioprotectants, surgery plus chemotherapy and immunity plus chemotherapy.

V. Conduct of international activities including maintenance of chemotherapy offices in Europe and Asia, arrangements for acquisition of test materials, screening of new agents abroad and surveillance for and acquisition of new antitumor agents.

This program is planned so that it will be highly responsive to the needs of the preclinical and clinical chemotherapy program of the Cancer Institute. It maintains close relationships with medical schools, pharmaceutical houses, university and research institutes both in the United States and abroad in areas pertinent to chemotherapy.

Methods Employed: Standard laboratory procedures were employed.

Relationship of Preclinical Investigations of Antitumor Agents to the Clinic:

Dr. Ira Kline, serving as an Expert Consultant in the Drug Research and Development Program, is the principal investigator in this program which is conducted in collaboration with S. K. Carter, J. M. Venditti, F. Muggia, and M. Slavik.

Cooperating units include: M. Rosenzweig, Investigational Drug Branch; A. Guarino, R. Davis, Experimental Therapeutics, DCT, NCI; Staff of the Drug Evaluation Branch, DCT, NCI; H. B. Wood, Jr., and staff, Drug Development Branch, DCT, NCI; I. Miller and S. Stone, Arthur D. Little; and the DCT-NCI-Contract-Screening Laboratories.

Good progress is being made in this program as follows:

I. New Test Systems Other Than the Ones Currently Employed for the Identification of New Antitumor Agents

During the period of this report, there was participation in Program Planning Meetings chaired by the Associate Director for DR and DP. The advisability of using the P-388 leukemia as an *in vivo* prescreen was reviewed and was adopted for this purpose in the screening program. The biological characteristics and cost feasibility of various new tumor systems were investigated, and selected systems currently are being incorporated into the screen. The systems include various human xenografts propagated in athymic mice, transplantable mouse colon, breast, and melanotic tumors along with mouse leukemia L1210.

II. Monitoring the World Literature for New Drugs for Acquisition and Testing

During the surveillance of the scientific literature, compounds with *in vitro* or *in vivo* antitumor efficacy or with other demonstrated biological activity were noted. With the assistance of the Drug Development Branch, searches of chemical structures were performed to determine the existance of the new agent in our program. For agents not present in the program, an effort is made to procure it. Approximately 10 new agents were detected and are being procured. Examples of these agents are: Analogs of bikaverin, 4-(β-D-ribofuranosyl)-1, 3-dihydroxybenzene, methioninyl adenylate, and an analog of Lapachol, 2-(3 methyl-2-butenyl)-3-(tetraacetyl-β-D-glucopyranosyloxy-1),4-naphthoquinone.

III. Summarize and Analyze Toxicity Data Derived from Small Animals and Man of Investigational New Drugs (IND)

As part of a program to study the correlation of tolerated doses in mice and in man, mouse toxicity data ($LD_{10,50}$ and $_{90}$) for 58 drugs given IP or IV via three schedules of treatment were assembled. Related data from clinical Phase I studies were also collected. After reviewing this information, it was decided to carry our more detailed toxicity studies in mice before continuing with our comparison, and such toxicity experiments currently are in progress. Other selected toxicity information from our animal data files was supplied to our colleagues of the Investigational Drug Branch for anticipated Phase I programs.

IV. Correlation of the Antitumor Activity in Experimental Animals and Man for Selected Agents

A preliminary analysis has demonstrated very well the feasibility of using confirmed screening results for 5 mouse tumor systems (L1210 ip, L1210 sc, P-388, B-16, and LL) to rank potential antitumor agents as candidates for clinical trial. The animal tumor data and related clinical data for 45 compounds were collected, interpreted and, in turn, given to our Contractor A. D. Little, for analysis. The results were encouraging with regard to the ability to predict clinical activity on the basis of animal test results. We are currently updating our clinical evaluations, adding new compounds, and are adding animal data for drugs currently in Phase I and early Phase II trials. With this additional information, we hope to derive additional in-

sight into the correlation of the animal systems and clinical trials.

V. Correlation of Synergistic Drug Combinations in Man and Animal Systems

As a follow-up of our correlation studies of the antitumor effects of single drugs in man and in animal systems, a related program was started to analyze the data for combination chemotherapy in man and in animals. The computerized animal data of the DED related to combinations active in man, effective human disease-oriented single drugs studied in combination, and the confirmed active combinations in animals, have been tabulated, and reviewed with our preclinical and clinical colleagues. Other related data are being assembled, and other specific combinations of clinical interest will be studied in animal systems to evaluate the correlation of the animal systems and man.

VI. Study Various Chemical Agents Tested in the Tumor Systems of the Joint U.S.A.-U.S.S.R. Program

A collaborative program has progressed for the interchange and evaluation of U.S.A. and Soviet potential antitumor agents. During the year, additional related testing was performed by our Contracting Laboratories. These data are currently being compiled and analyzed in anticipation of a joint meeting to be held in April, 1976 between representatives of the NCI and the U.S.S.R. This segment of the program has been functioning well, and four manuscripts related to these joint efforts are ready for publication.

VII. Historical Background of Drugs of Current Clinical Interest

Twenty-nine antitumor agents were selected which showed definite activity against at least one human hematological malignancy or solid tumor. Utilizing publications, information was collected related to the rationale or reason for the preparation of the individual agents. Also, for each agent, a list of in vivo or in vitro test systems in which the biological activity of the agent was detected, was prepared. A manuscript presenting this information has been written.

In addition to the above projects, Dr. Kline participated in and contributed to the scientific discussions and decisions of the DCT Operating Committee, Drug Evaluation Committee and Decision Network Meetings.

The following talks describing various aspects and results of our program were given by I. Kline:

1. Experimental Solid Tumors as Screening Systems. Presented at the Divisions of Nuclear Medicine and Radiation Health, Johns Hopkins University, Baltimore, Maryland.

2. Preclinical Studies with Potentiated Antitumor Drugs. Presented at the Kiwanis Club, Bowie, Maryland.

International Activities

There has been information exchange of most recent developments in preclinical and clinical chemotherapy, procurement of synthetic agents and natural products for screening and identification of new compounds of potential interest for chemotherapy.

Dr. Omar Yoder, serving as an NCI Division of Cancer Treatment officer at the European office, continues to make excellent progress in furthering the overall collaboration in Europe. The operation of the Liaison Office and Cancer Chemotherapy Research Laboratory at the Institut Jules Bordet which was established in 1972 continues to play an important role in the development of cancer research and treatment in Europe. In close collaboration with the European Organization for Research on Treatment of Cancer (EORTC) the operation continues to function as the most important link between cancer research and treatment in Europe and the National Cancer Institute in the United States. Dr. Yoder was assigned the task to take charge of the Office in Brussels. A full-time multilingual secretary continues to assist in the daily activities of the Office.

The European Organization for Research on Treatment of Cancer (EORTC) was founded in 1962 by the main cancer research institutions of the Common Market countries and Switzerland. Representatives of Great Britain and Austria joined the Organization in 1966 and 1970, respectively. Since that time, efforts have been made to extend the boundaries for associate memberships to representatives from Eastern Europe. The EORTC has a Scientific Council which plans and supervises activities within the Organization. Dr. Yoder is a de facto member of the Scientific Council and a member of the EORTC Screening and Pharmacology Group which reviews all potential new drugs for clinical trial within the EORTC. The Administrative Board of the Council handles the daily work involved with administrative and financial matters. Both clinical and laboratory work is carried out by the 34 separate units which are composed of clinical cooperative groups, project groups, clubs, working parties and task forces. The EORTC was recently recognized by WHO as one of its regional institutions. Under the leadership of Prof. H. J. Tagnon who was elected President in January, 1975, a number of significant events have taken place which should be viewed with optimism for the future of the EORTC. A European Foundation was created and is now beginning to function in order to give the EORTC the sound financial backing it needs to effectively accomplish its mission. The Foundation is now functioning under the leadership of Mr. Grierson, a London banker. The Council of the Foundation will consist of prominent personalities in the various member countries. They have committed themselves to collect a certain portion of the projected funds for the next three years. It is hoped that in three years the EORTC will be entirely supported from European sources.

The establishment of a Data and Coordinating Center was also made possible thru the initiatives of Prof. Tagnon who visualized its importance a few years ago in view of the projected expansion of the EORTC activities. This was made possible thru support from the NCI which will continue until the end of 1978. The Data Center is directed by Dr. M. Staquet (also EORTC coordinator)

who has a supporting staff of nine with the following composition: a statistician, a computer analyst, a programmer, three data-managers, two secretaries and a data-clerk. The Data Center provides supporting services to the various EORTC groups involved in clinical trials. Up to the present time, the Data Center has handled over 3,000 cases with participation of the major cooperative groups within the EORTC. A Protocol Review Committee, with representatives from the United States as well as from Europe, reviews all new protocols before a clinical trial is initiated within the EORTC. In September of 1975, a Breast Cancer Task Force was created, which was inspired by the one in the United States. Ten EORTC publications appeared in the medical literature during 1975. It can be clearly stated that the recent developments, which may be viewed as significant for the future of the EORTC, would not have been possible without the moral and financial support from the NCI over the past few years. The establishment of the NCI Collaborative Office in Brussels was also a major factor in influencing these events since it focused public attention in Europe on the importance of cancer research and treatment as a multinational problem.

A screening program continues to function successfully at the Mario Negri Institute in Milan for the testing of compounds collected primarily in Southern Europe. A chemotherapy program is in progress at the Chester Beatty Institute in England and we are collaborating with a screening program at the Cancer Institute in Dublin, Ireland. A program on plant collection, extraction and screening at the Weizmann Institute in Rehovoth, Israel is collaborating with us under a U.S.-Israel bilateral agreement has uncovered several materials of interest. The screening program at the Cancer Institute in Tokyo as part of the "Anticancer Drug Evaluation Center" is making progress in the testing of candidate agents. The materials that are being tested in the screens are being collected primarily from pharmaceutical houses and universities in Japan. The data collected in the screening programs in both Europe and Asia are entered into the Drug Research and Development computer program. The DCT offices in both Brussels and Tokyo continue to publicize extensively the DCT program in the various countries of Europe and Asia.

The program at the Radiological Institute in the Netherlands on the combination of radiation and chemotherapy is proceeding well.

The collaborative chemotherapy program under the U.S.-U.S.S.R. Cooperative Medical Agreement continues to function in exchange of information and for the expediting of the overall collaborative effort. A collaborative program has been in progress for the interchange and evaluation of Soviet and American drugs. Under the U.S.-Japan agreement a meeting was held on preclinical and clinical aspects of cytosine arabinoside and cyclocytidine.

Visits were made by A. Goldin as follows:

1. Participation and presentation of papers at the First Symposium on Chemotherapy as part of the U.S.-Japan Cooperative Cancer Research Program. The symposium was composed of two parts: a) A workshop on the anticancer drug cytosine arabinoside and the structurally related congener cyclocytidine, and b) A workshop on Phase I studies of anticancer agents. The U. S. delegation consisted of Drs. S. K. Carter, I. Krakoff,

D. Ho, K. McRedie, and A. Goldin.

2. A visit was made to the Institut Jules Bordet in Brussels where under the leadership of Prof. Henri Tagnon a strong drug development program has been instituted which is making significant input to the cancer chemotherapy program at the NCI. During this visit there was a review of the ongoing program under our liaison contract in discussions conducted with Professor Tagnon, Dr. Staquet, Dr. Kenis, Dr. Attasi, and with Dr. Yoder who is our representative at the European Office. Our program there has been highly active in the screening and subsequent evaluation of antitumor compounds and several of these are proving of definite clinical interest.

Participation and presentation of a paper in Brussels at a tumor immunology symposium. At this symposium latest developments were reviewed in immunology, immunotherapy and immunochemotherapy of cancer with participation of leading investigators abroad with expertise in these areas.

3. A visit was made to Bratislava in Czechoslovakia with participation in the International Union against Cancer (UICC/CICA) study group on drug resistance and selectivity. A paper was presented at this study group program and a report of the workshop is being prepared for publication. The loss of selectivity accompanying the phenomenon of tumor cell resistance is a primary limiting factor to the successful outcome of chemotherapy both at the preclinical level and in the clinic. The primary objective of the workshop was, therefore, to discuss target cell determinants that could influence antitumor effectiveness of drugs and the extent of drug resistance. The possibility exists that the identification of target determinants of drug action may serve as indicators of the sensitivity or resistance of individual clinical tumors to the available antitumor agents. It was considered that in depth focus on specific areas and problems would serve as a basis for eventual identification of the multiplicity of factors that may influence sensitivity or resistance. An important recommendation was made that CICA organize a workshop on "Methodology of human tumor sampling for biochemical-pharmacologic studies of target determinants of drug action." The recommendation was also made that the Geneva office of CICA prepare, a) bibliographies on methodology pertaining to the handling of tumor samples, b) a publication on the biological basis of clinical resistance.

In Milan, visits were made a) with Professors Ghione and Arcemonie and staff at Farmitalia for the discussion of their synthesis program on adriamycin and daunomycin analogs, b) with Professor DiMarco and staff at the Italian National Cancer Institute for discussions on the biologic and antitumor properties of new adriamycin and daunomycin derivatives and collaborative investigations, c) with Drs. Garratini and Spreafico and staff for discussion of contract activities and collaborative research, and d) with Dr. Enzo Bonmassar of the Pharmacology Department, Perugia Medical School and Dr. Angelo Nicolin of the Pharmacology Department at the University of Milan Medical School concerning manuscripts in preparation and collaborative investigations.

Participation and presentation of a paper at the 9th International Con-- gress of Chemotherapy in London. Specific participation was at a symposium on combination chemotherapy.

A visit was made to Dr. Michael Conalty and his colleagues at the Laboratories of the Medical Research Council of Ireland, Trinity College, Dublin. Dr. Conalty has been engaged in cancer research for many years and has been submitting new compounds to our program. At this laboratory there is a vigorous cancer research program in which chemists and biologists are cooperating closely. Several classes of compounds have shown activity in animal tumor systems.

4. Participation and presentation of papers at a WHO meeting in Milan on Rational Design Synthesis of Compounds for Cancer Chemotherapy. This meeting was supported by a grant from the National Cancer Institute, Bethesda, and was held at the Institute Mario Negri in Milan. The proceedings of this program are being published. Participation in the meeting was by leading investigators on a worldwide basis.

5. Participation in a symposium on Design and Achievements in Chemotherapy in honor of Dr. George H. Hitchings at the Burroughs-Wellcome Laboratories.

6. Participation and presentation of a paper and chairing of a session at an International Conference on Immunobiology of Cancer sponsored by the New York Academy of Sciences; also participation at an International Conference of Immunotherapy of Cancer, also sponsored by the New York Academy of Sciences. The programs were of specific interest particularly in view of the stress being placed at the current time in the combined modality of immunity plus chemotherapy in the treatment of cancer.

The following talks describing various aspects and results of our program were given at international conferences during the year by A. Goldin:

1. The Potential Impact of Experimental Studies on the Clinic. Presented at the U.S.-Japan Joint Workshop, Tokyo.

2. Conclusion and closing remarks to A Comparative Study on Cytosine Arabinoside and Cyclocytidine at U.S.-Japan Joint Symposium in Tokyo.

3. Factors Pertaining to Drug Uptake and Selectivity. Presented at a study group on drug resistance and selectivity in Bratislava, Czechoslovakia under the auspices of the International Union Against Cancer.

4. Relationship of Structure to Antitumor Activity for Folic Acid Antagonists. Presented at a meeting on "Rational Design and Synthesis of Compounds for Cancer Chemotherapy" held under the auspices of WHO at the Mario Negri Institute in Milan.

5. Separation of Antitumor Activity and Host Toxicity. Presented at the meeting on "Rational Design and Synthesis of Compounds for Cancer Chemotherapy" under the auspices of WHO in Milan.

6. Perspectives of Immuno-chemotherapy. Presented at the symposium on Human and Clinical Tumor Immunity at the Annual EORTC Symposium at the Institut Bordet in Brussels.

7. Influence of Immune Modulators on the Oncogenic Process. Presented at the International Conference on Immunobiology of Cancer under the auspices of the New York Academy of Sciences.

8. The Biological Basis of Combination Chemotherapy. Presented at a symposium on combination chemotherapy at the 9th International Congress of Chemotherapy in London.

Major Findings

I. Uptake of 5-Iododeoxyuridine as a Measure of Tumor Growth and Tumor Inhibition (with E. Bonmassar, D. P. Houchens, and M. C. Fioretti)

Growth of generalized tumors such as spontaneous or transplanted lymphomas or other rapidly metastasizing neoplasms is not too easily evaluated when the host is either untreated or subjected to antitumor therapy. The usual parameters of response have been measurement of the life span of the animals and to some extent the bioassay of the tumor bearing animals. In this study tumor cell proliferation in various organs and peripheral blood was evaluated by the extent of DNA synthesis as reflected in the uptake of 5-iododeoxyuridine labeled with iodine-125 (^{125}IUdR). A direct relationship is demonstrated between isotope uptake values and the extent of neoplastic growth. The ^{125}IUdR uptake method was determined to be applicable to measurement of inhibition of tumor growth in drug treated animals and in pre-sensitized allogeneic hosts. DNA synthesis of tumor cells was determined in spleen, lung, and liver of mice bearing spontaneous or transplanted lymphomas or Lewis lung carcinoma. Also, an in vitro uptake method was developed to assess ^{125}IUdR uptake of peripheral blood of leukemic animals. Thus, the studies suggest that ^{125}IUdR uptake may be applicable to monitoring tumor growth in vivo and for the evaluation of the inhibitory effects of antineoplastic chemotherapy or antitumor immunity.

II. Increased Immunogenicity of Two Lymphoma Lines after Drug Treatment of Athymic (nude) Mice (with F. Campanile, D. P. Houchens, M. Gaston, and E. Bonmassar)

It was demonstrated that highly immunogenic sublines of L1210 and LSTRA lymphomas could be obtained from athymic (nude) mice treated with DTIC in vivo. Conventional mice compatible with the parent tumor rejected the DTIC treated sublines and were relatively resistant to a subsequent challenge with the parental lines. On the other hand, the DTIC treated sublines were not rejected by athymic mice, indicating that the transplantation resistance to these tumors in conventional mice was thymus cell dependent. The study suggests the possibility of obtaining sublines of human tumors with similar properties in athymic mice. If the immunogenic properties of human tumors can be modified by drug treatment in vivo in athymic mice the model might provide a basis for a clinical immunotherapy approach.

III. Immunochemotherapy of Transplantable Moloney Leukemia with Cyclophosphamide and Allogeneic Spleen Lymphocytes (with M. Kende, L. Keys and M. Gaston)

When histoincompatible spleen cells sensitized with Moloney Sarcoma Virus were used in conjunction with an immunosuppressive chemotherapeutic dose of cyclophosphamide in the treatment of transplantable Moloney Virus induced leukemia, fatal graft vs. host disease occurred in BALB/c mice. When antiserum against the donor spleen cells was administered two days after immunochemotherapy, graft vs. host disease was prevented, but no immunotherapeutic effect was observed. When the antiserum was delayed for three days or more lethal graft vs. host disease occurred. Utilization of Moloney Sarcoma Virus sensitized BALB/c x C57Bl/6 F_1 spleen cells in conjunction with cyclophosphamide "cured" a high percentage of treated mice. When allo-antiserum was administered two days after immunochemotherapy the immunotherapeutic effect was abolished. However, the allo-antiserum was not able to reverse the immunotherapeutic effect three days post grafting or later and there again resulted a high percentage of long term survivors. The importance of timing relationships in attempts to control graft vs. host disease with retention of antitumor effectiveness is emphasized by these studies.

IV. Preclinical Characterization of Candidate Antitumor Drugs (with R. K. Johnson and J. M. Venditti)

This is a comprehensive review of methodology for further evaluation of new compounds, following detection of activity in a primary antitumor screening program. A major objective of a preclinical program is to characterize activity of the new compound with respect to parameters that have relevance for the clinic. The review, although not exhaustive, illustrates the types of systems currently available for drug characterization following the initial demonstration of activity. New compounds could be characterized in a relatively rapid systematic manner in accordance with planned protocols to provide a broad base of data for decision making for further drug development and possible clinical investigation. These planned protocols can follow the same format and serve as an extension of the "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and other Biological Systems" (R. Geran, N. H. Greenberg, M. M. Macdonald, and B. J. Abbott, Cancer Chemotherapy Reports, Pt. III, Vol. 3: 2-103, 1972). The systems discussed include in vivo pharmacologic testing, drug activity in a spectrum of tumor systems, in vivo antitumor testing of drugs, in vitro characterization of drugs and application of methodology to selection of analogs.

V. Preclinical Approaches in Tumor Immunochemotherapy (with D. Houchens)

This is a review stressing approaches in immunochemotherapy. Areas are outlined in which there have been illustrations of an increased therapeutic response on treatment with a combination of immunotherapy plus chemotherapy. In one instance, for example, an immune response to a sensitive line of leukemia L1210 was capable of improving the therapeutic response against a resistant line of leukemia. In another example, moderate preimmunization of the animal against a leukemic line improved chemotherapeutic response to chemo-

therapy. Adoptive immunologic procedures have been capable of improving therapeutic response to a drug for not only leukemia L1210 but also for a transplantable Moloney leukemia as well as a primary Moloney Sarcoma virus induced sarcoma. Progressive therapy with a drug may result in an alteration of the antigenicity of the tumor cells and these cells may be protected by the origin of drug resistance. The presence of antigenically altered cells may in turn lead to improved therapeutic response to a second drug. There would appear to be some specificity in the origin of this new antigenicity. The ability to alter tumor cell antigenicity in athymic mice suggests that it may be possible to alter antigenicity of human tumors in these mice and taking advantage of residual common antigen to employ such tumors in immunotherapeutic investigations. There has been considerable emphasis over many years in chemotherapy and emphasis and interest in immunotherapy. The approaches and studies currently reviewed suggests that the two modalities may be combined profitably in the form of immunochemotherapy for the improvement of therapeutic response. There has been only an initial modest start in this direction employing animal model systems.

VI. Cellular Immune Response to a Drug Treated L5178Y Lymphoma Subline (with A. Nicolin, A. Bini, and E. Coronetti)

In earlier studies in this laboratory it was shown that it is possible to increase the immunogenicity of tumor cells. Immunogenic L1210 sublines were induced by prolonged in vivo treatment with DTIC. Mice compatible with the untreated L1210 leukemia were resistant to a heavy challenge with L1210/DIC cells, whereas host resistance could be abrogated by immunodepression. The alteration of antigenicity acquired by L1210 treated cells (L1210/DIC) was found to be heritable and in the current investigation it was shown that a subline of L5178Y tumor transformed by DIC in vivo (L5178Y/DIC) implanted into mice compatible with the parental leukemia stimulated a specific cell mediated cytotoxic response.

The remainder of the major findings in collaborative program are summarized in the annual reports of the Tumor Biology and Immunochemotherapy Sections of the Laboratory of Experimental Chemotherapy and in the annual reports of the Drug Research and Development Branches.

Significance to Biomedical Research and the Program of the Institute

This project contributes to the planning and coordination of the chemotherapy and combined modality research program of Drug Research and Development and to interdigitation with the preclinical and clinical programs. It serves as a focal point for coordination and collaboration with relevant programs in other institutions both in the United States and abroad. It provides a mechanism for collaborative investigation of special problems of program interest.

Proposed Course of the Project

The various aspects of the project will be continued and modified in accordance with laboratory findings and clinical interest. Continued emphasis will

be placed on developing the international program and world surveillance for drugs for screening, drugs of interest for the clinic and new modalities of therapy. Continued emphasis will be placed on activities related to the planning and coordination of the total DR&DP research program and on the further development of the preclinical-clinical interrelationships with respect to the predictability value of animal test models, combination chemotherapy and combination modalities. It is planned to develop further collaborative investigations of the Drug Research and Development Program with the preclinical and clinical groups of the Division of Cancer Treatment as well as collaborative research in the United States and abroad.

Publications

1. Woodman, R. J., Cysyk, R. L., Kline, I., Gang, M, and Venditti, J. M. Enhancement of the effectiveness of daunorubicin (NSC-82151) or adriamycin (NSC-123127) against early mouse L1210 leukemia with ICRF-159 (NSC 129943). Cancer Chemother Rep. 59: 689-695, 1975.

2. Bonmassar, E., Houchens, D. P., Fioretti, M. C., and Goldin, A. Uptake of 5-iododeoxyuridine as a measure of tumor growth and tumor inhibition. Chemotherapy 21: 321-329, 1975.

3. Campanile, F., Houchens, D. P., Gaston, M., and Goldin, A. Brief communication: Increased immunogenicity of two lymphoma lines after drug treatment of athymic (nude) mice. J. Natl Cancer Inst. 55: 207-209, 1975.

4. Kende, M., Keys, L., Gaston, M, and Goldin, A. Immunochemotherapy of transplantable Moloney leukemia with cyclophosphamide and allogeneic spleen lymphocytes and reversal of graft-versus-host disease with alloantiserum. Cancer Res. 35: 346-351, 1975.

5. Bonmassar, A., Fioretti, M. C., Bonmassar, E., and Goldin, A. Allograft reactivity of mice against a radiation-induced lymphoma incompatible for the H-2K-Ir regions of H-2. Europ J Cancer 11: 71-78, 1975.

6. Bonmassar, E., Campanile, F., Houchens, D., Crino, L., and Goldin, A. Impaired growth of a radiation-induced lymphoma in intact or lethally irradiated allogeneic athymic (nude) mice. Transplantation 20: 343-346, 1975.

7. Goldin, A., Johnson, R. K., and Venditti, J. M. Preclinical Characterization of candidate antitumor drugs. Cancer Chemother Rep. 5: 21-81, 1975.

8. Goldin, A., and Houchens, D. P. Preclinical approaches in tumor immunochemotherapy, in press.

9. Nicolin, A., Bini, A., Coronetti, E., and Goldin, A. Cellular immune response to a drug-treated L5178Y lymphoma subline. Nature 251: 654-655, 1975.

10. Goldin, A., and Johnson, R. K. Experimental tumor activity of adriamycin (NSC 123127). Cancer Chemother Rep. 6: 137-145, 1975.

Appendix A

Office of the Associate Director
Laboratory of Experimental Chemotherapy
Contracts

Contract # NO1-CM-53673

Principal Investigator: Dr. L. M. Van Putten

Radiological Institute, TNO, Rijswijk (The Netherlands)

Objectives:

To develop experimental models and provide information of great importance to the DCT program in the area of combination radiation and chemotherapy.

Major Findings:

In its first year of operation, this program has been highly successful. The experimental studies under this contract on the effects of combined radiation and drugs fall in three primary categories: a) Studies on optimal application of a phase specific drug such as vinblastine at an optimal time interval after radiation in order to take advantage of the recruitment of resting cells by the preceding radiotherapy; b) Studies on a number of cytotoxic drugs applied simultaneously with radiation; c) Studies with hypoxic radiosensitizers.

In studies on tumors in rats, several subjects were investigated: a) Growth delay of the R-1 rat rhabdomyosarcoma after combined treatment with x-rays and vinblastine. The combination of 1.5 mg/kg of vinblastine plus 1000 rad of x-rays gave an excess growth delay of more than 8 days as compared with individual modalities. This excess growth delay was approximately equal to the value of 7.5 days excess delay derived from the combined treatment with 2000 rad of x-rays and 1.5 mg/kg of vinblastine. In this study the excess growth delay is defined as the difference between the delay obtained in the combined treatment and the sum of the delays of the individual treatment with 1000 rad of x-rays or 1.5 mg of vinblastine alone. b) Growth delay of the RSC rat squamous cell carcinoma after combined treatment with x-rays and vinblastine. In order to determine whether the enhancement of effectiveness of vinblastine due to prior administration of doses of ionizing radiation to R-1 rat rhabdomyosarcoma can also be demonstrated for other types of tumors, experiments in growth delay were carried out with a rat skin baso-squamous cell carcinoma. In this investigation a dose of 2000 rad of x-rays caused a long growth delay of 110 days. Vinblastine alone, however, was essentially ineffective and the excess delay in tumor growth with the combination of irradiation plus vinblastine was negligible. From this it could be concluded that the enhancement of the effectiveness of vinblastine by preceding irradiation is not a general phenomenon for all types of tumors.

Studies were conducted on the combined effects of simultaneous administration of drugs and fast neutrons on normal hematopoietic stem cells. These studies with neutrons indicate a marked enhancement of effect of vinblastine when each modality is applied at doses which when given alone cause a similar decrease in cell survival as used for the gamma ray studies. It would appear that the potentiation by the combination of radiation and vinblastine can occur independently of radiation quality and independently of the proliferation rate of the stem cells. It was also observed that the greater than additive effect of 5-FU after radiation, which is usually ascribed to a block of repair, is still quite evident also after neutron irradiation.

In addition, studies were performed on the effect of dose of both vinblastine and radiation on the degree of potentiation. It was found that a more than additive effect is obtained only after the relatively high dose of 15 mg/kg of vinblastine.

Studies were also performed with a series of other agents in combination with 200 rad gamma irradiation. Enhancement was observed with 5-fluorouracil and with ftorafur but not with other agents such as vincristine, daunorubicin, adriamycin, bleomycin, VN26VP16, metbotrexate, Ara C, procarbazine, or hycanthone. CCNU appeared to give moderate enhancement.

The effectiveness of combined treatment against leukemia L1210 was determined as follows: Mice were injected intravenously with 10^4 L1210 cells from an in vitro culture. After 6 days groups of 5 of these leukemic mice were exposed either to radiation alone or cytostatic drug alone or the combination of radiation and a chemotherapeutic agent. This system has been studied so far with a limited number of drugs. No enhancing effect of vinblastine could be observed with the combination of vinblastine plus x-ray. This markedly greater than additive effect was noted. The data suggests that sublethal damage caused by radiation and that caused by adriamycin may interract to produce cell lethality.

Studies were conducted with hypoxic sensitizers on poorly reoxygenating male osteosarcoma. The 2-nitroimidazole compound Ro-07-0582 demonstrated radiosensitizing effects when given during one week of radiotherapy with small daily fractions (5 x 300 rad per day) but not when given in combination with 2, 3 or 4 weeks of radiotherapy using this fraction schedule. This has led to further studies in two areas. a) The possiblity of inactivation of the sensitizers in microsomal liver enzymes was studied by combining treatment with SKF525A, a blocker of these enzymes. There did appear to be a temporary difference between the groups treated with and without SKF525A. However, the terminal regrowth curve indicated no difference. This result could be interpreted to mean that the apparent reversal of early sensitizing effects cannot be attributed to more rapid metabolism of the sensitizer. b) Effect of the sensitizer in this tumor system was applied in a more widely spaced fractionation scheme using larger doses per fraction. The effectiveness of therapy was clear when the sensitizer was given with each of the 6 radiation fractions applied. But it would appear that the sensitization of the first 3 fractions may be more important. This data, although preliminary, would suggest that for clinical application of this sensitizer the use of fewer larger fractions may be preferable. A preliminary report on this subject is to be published.

In the clinical activities for the treatment of acute myeloblastic leukemia the protocol CCGAL-1, a combination of DMP (daunomycin, cytosine arabinoside, 6-MP and prednisolone) is being compared with DCMP (daunomycin, cyclocytidine, 6-MP and prednisolone).

In another protocol for acute myelocytic leukemia the combination of NCVP (neocarzinostatin, cytosine arabinoside, vincristine and prednisolone) is being employed.

For non-Hodgkins lymphoma, VCP (vincristine, cyclophosphamide and prednisolone) is being utilized.

For Hodgkins disease a protocol involving a combination of vincristine, cyclophosphamide, procarbazine and prednisolone is being planned.

A total of 68 patients (both (in-patients and out-patients) was treated in the clinic of the Division of Clinical Chemotherapy.

Information Activity:

497 titles of abstracts were sent to NCI and 125 of these were translated at our request.

Reports:

There were 7 of the annual meetings, 13 of the group study meetings, 2 of the 34th Annual Meeting of the Japanese Cancer Association and 5 reports of latest data on ACNU, GANU, 40497-S, N-acylcytosine arabinoside and maruyama vaccine.

The folowing compounds recently developed in Japan have been sent for testing at NCI: 4-hydroperoxy isophosphamide (40497), ACNU, GANU, maruyama vaccine.

For purposes of maintaining close contact and for exchanging information regarding recent developments in preclinical and clinical cancer chemotherapy trips were made to the USA, Nishinomiya Fukuoka, Kyoto, Nagoya, Osaka and TSU. Overall the liaison office program in Tokyo is proceeding well particularly at the preclinical level. At the clinical level the program is beginning to show some momentum.

Significance to Biomedical Research and the Program of the Institute:

This project in Japan is necessary in order to contribute to a continuing flow of new compounds and materials into the Division of Cancer Treatment for further developments in preclinical and clinical chemotherapy.

Proposed Course of Project:

To continue the development and flow of new drugs and materials between the U.S. and Japan.

MAMMALIAN GENETICS AND ANIMAL PRODUCTION SECTION

INTRODUCTION

The primary function of the Mammalian Genetics and Animal Production Section is to provide healthy laboratory animals with properly defined genetic characteristics to various research investigators as follows:

1. DR&D Screening Program.

2. Other NCI Research Contracts.

3. Research Grants (strains not available commercially and surplus animals which are offered when available).

4. Veterans Administration Research Facilities (surplus animals which are offered when available).

5. NIH Intramural Program

A secondary function of this Section is to coordinate the movement of transplantable experimental animal tumors from tumor bank sources into scientific laboratories.

The structure of the animal production program includes:

4 Primary Genetic Centers

4 Genetic Production Centers

6 Rodent Production Centers

12 Hybrid Production Contracts

3 Beagle Production Contracts

3 Diagnostic Contracts

2 Histocompatibility Study Contracts

2 Contracts with the National Academy of Sciences

The Section staffing consists of one senior staff member, one professional associate, one animal contracts assistant, and one clerk-typist. Also assigned to this Section is one animal caretaker supervisor and 4 animal caretakers who function in DR&D in-house laboratories.

The overall production of these facilites supplied the following strains and numbers of laboratory animals during calendar year 1975.

Mice	
Inbred weanlings	805,555
Inbred pregnants	2,040
Inbred litters	260
Hybrid Weanlings	3,207,078
Hybrid pregnants	86
Outbred mice	27,564
Outbred pregnants	10
Rats	
Inbred weanlings	57,312
Inbred pregnants	93
Inbred litters	7
Outbred weanlings	10,447
Outbred pregnants	9
Outbred litters	0
Hamsters	
Weanlings	17,150
Pregnants	1,241
Beagles	
	585

The various animal supply contracts are structured as indicated:

PRIMARY GENETIC CENTERS

These centers serve as a breeding nucleus for the animal program. In order to insure that maximum quality control is maintained at this level, it is required that any animals received by such centers are derived into a germ-free state; i.e., Caesarian sections are performed under isolator conditions. These animals are then maintained as foundation colonies in defined flora isolators. These foundation isolators serve as a source of breeders for the larger expansion colonies which are housed uder barrier conditions.

GENETIC PRODUCTION CENTERS

Foundation animals for these centers are received from isolator colonies via germfree shippers and are placed directly into the barrier-maintained facilities for propagation. Otherwise, the same quality control standards are maintained as described for the primary centers.

RODENT PRODUCTION CENTERS

The purpose of these contracts is to accomplish large-scale production of pure strains of inbred mice which will be supplied to fixed-price contractors for hybrid mouse production. The rodent production contract receives

breeding stock from the genetic center expansion colonies. These breeders, therefore, represent direct offspring of brother x sister matings. This production also takes place under rigid barrier-controlled conditions.

HYBRID MOUSE PRODUCTION CONTRACTS (FIXED PRICE)

These contractors supply the large volume of first generation hybrid mice for the screening program. Breeding animals for these colonies are received from the rodent production contracts. Animals are propagated under semi-barrier conditions.

BEAGLE PRODUCTION CONTRACTS

These animals are procured on a fixed-price contract basis from qualified breeder and are supplied primarily to the toxicology program.

DIAGNOSTIC CONTRACTS

These are service contracts which are utilized to monitor and upgrade the quality of animals moving within the animal production program and to the laboratory. Presently, they consist of one virus diagnostic service, one parasite diagnostic service, and one bacterial diagnostic service (primarily Salmonella and Pseudomonas species). Additionally, a limited but very significant contribution to the laboratory animal disease diagnosis program has been performed by Dr. Russell Lindsey of the University of Alabama. Dr. Lindsey has performed gross and micropathology services upon request without funding. We are presently negotiating for a pathology services contract which will be a very important asset to our quality control program.

HISTOCOMPATIBILITY STUDY CONTRACTS

These contractors perform genetic monitoring of inbred mouse and rat strains by means of skin grafts.

NATIONAL ACADEMY OF SCIENCES

A small amount of support via Task Orders 6 and 16 is supplied to the National Academy of Sciences. This support involves develooment and publication of standards and guidelines in the field of animal care and breeding. In addition, support is extended for holding conferences and meetings pertaining to the field of laboratory animal care.

DISCUSSION:

The Mammalian Genetics and Animal Production Section is utilizing the Small Animal Section of the Veterinary Resources Branch as a repository for inbred rodent sublines of NIH origin. Pedigreed litters have been supplied to our genetic centers from this section and will continue to be supplied on a regular and continuing basis. It is felt that in this manner we can reach and maintain a degree of genetic uniformity with our inbred and hybrid strains that was not possible previously when each genetic center was

propagated entirely from its own breeding stock which in effect meant that each center developed its own subline with consequent genetic drift.

In cooperation with Dr. Carl Hansen of the Veterinary Resources Branch we are searching for a more sensitive method of testing for histocompatibility with both the reference mice (NIH) and within our own colonies. In the interim we will continue reciprocal skin grafting using the NIH colonies as reference material.

We have also carried out life span studies with L1210 leukemia implanted in BDF_1 mice which originated from inbred strains obtained from NIH. Life span data from those studies which involved several titrations (10^7-10^1) including approximately 2,000 mice indicated that these animals with the NIH breeding were quite satisfactory for this tumor. Inexpensive studies of this type when screening tumors are injected in the proper host with known NIH breeding should help to maintain our confidence in the NIH repository system.

We feel that progress is also being made in our Animal Health Program. Primarily genetic centers have been "closed" to the extent that no animals enter except through Caesarian derivation. This policy has been maintained for approximately one year during which time foundation colonies with several inbred strains have been established by brother x sister matings within isolators. Larger expansion colonies are being established from isolator animals with defined flora. These animals are maintained under strict barrier conditions. Super-clean animals are now becoming available on a limited basis and are being supplied to selected rodent production centers. We anticipate that these centers will in the not too distant future be able to supply large numbers of clean healthy inbred strains for hybrid production and for laboratory usage.

We have recently awarded a contract for the production of a sizable number of athymic (nude) mice for the screening program. These mice will be produced under the same strict barrier conditions that are required for the genetic centers. It is essential that these animals be produced and utilized at the laboratory uder extremely aseptic conditions because of their unique susceptibility to disease.

All laboratory animals are monitored for viral, bacterial, and parasitic diseases. We are presently negotiating for a pthology services contract which will be vary important to us in determining causative organisms when disease outbreaks occur in the laboratory or in the mouse colony.

As the production of clean inbred strains and hybrids become available (a small number of clean hybrids are available now) we feel that the receiving laboratories should re-examine their own holding and experimental facilities. Most of our screening laboratories will need to upgrade their facilities before they can take advantage of these clean animals in experimental programs.

ANNUAL REPORT

DRUG DEVELOPMENT BRANCH

DRUG RESEARCH AND DEVELOPMENT

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

Because cancer is not one disease but a number of diseases affecting the different organs and systems of the body, many different approaches to cancer treatment may be adopted. The major approaches include surgery, radiation, chemotherapy, immunotherapy, or a combination of the four. Research in drug development includes selection and procurement of agents for testing, bioassay of the agent in tumor systems, preclinical pharmacological and toxicological study, and clinical evaluations. The foundation of chemotherapy is the drug itself - either single or in drug combination. Consequently, the search for more effective, less toxic and broader acting agents will continue.

Past experience has clearly demonstrated that effective antitumor agents are not restricted to a single, or even a few, chemical classes but that the chemical-structural parameters are broad. One must conclude then that all effective agents are not operating by a single mechanism. Therefore, efforts are directed towards the chemical modifications of existing drugs to increase their activity and/or decrease their toxicity, and to search for new agents via chemical synthesis, or from natural sources. The latter category includes higher plants, marine invertebrates, insects, other animals, algae and fermentation filtrates. Materials from these sources will be evaluated in the primary screen and those agents demonstrating sufficient activity will be subjected to toxicological studies and possible clinical evaluation.

Research efforts are, in large measure, based on leads provided by the primary screens, biochemical and toxicological studies, pharmacokinetics observations, literature reports, and the surveillance of the worldwide scientific community for information on new drugs. As the vast storehouse of information accumulates and greater achievement is being experienced in the control of the more rapidly proliferating phase of cell growth or young tumors, the search for new structures aimed against the slower growing tumors in specific organs, i.e., lung, breast, colon, brain, kidney, etc. will be increased.

Objectives

As part of the broad Division of Cancer Treatment Program, the Drug Development Branch has three major objectives:

I. The Synthetic Drugs

The acquisition of new synthetic materials via contract synthetic program and the acquiring of new synthetic materials from the scientific community

at large are being undertaken. Fundamental chemical research on the theoretical design of new structures via structure-activity evaluations in collaboration with investigators of biochemistry, biology, toxicology, pharmacokinetics, and clinical trials is utilized. A computer data bank for chemical drug information is being developed, maintained and utilized. Research is also in progress towards the preselection of chemicals for the screen.

II. The Natural Products Drugs

The acquisition of new materials from natural sources - plant, fermentation and marine products is the major function of this area. A contract program in the natural products areas centers around extraction, isolation and identification of new materials. Complimentary to this program is the systematic collection of plant materials, soil samples and marine life for the investigative search of active antitumor agents. Crucial to this program is the systematic identification of the natural product being explored.

III. The Applied Services

The two major services are the preparation or resynthesis laboratories for bulk and natural products materials for secondary screening, toxicology and clinical trials, and the clinical dose formulations, both oral and parenteral for use in man. Complementary to the two major activities are three minor services - the quality control activity designed to evaluate bulk chemicals and formulated drugs as to their identity, assay and purity prior to use in man; the preparation of radiolabeled drugs used in advanced biology, toxicology or clinical investigation for metabolism, distribution and mechanism of action studies; and the storage and distribution of clinical dosage forms to clinical investigators.

Implementation of Program

These objectives are carried out through the activities of the Branch's Sections which are:

I. Chemical and Drug Procurement Section: The objectives are divided into the synthesis, preparative, and quality control activities. The synthesis activities are involved with the synthesis of new compounds of diverse structures for antitumor evaluation. As the rapidly growing tumors become more and more under control, attention will be focused upon developing new drugs for the slower growing tumors, especially for lung, breast, colon, etc.

The preparative activities include the process development and improvement of procedures for large scale production; the synthesis and isolation of large quantities of bulk chemicals and drugs for confirmational testing, pharmacological and toxicological evaluation, and clinical studies; and preparation of high purity radiolabeled materials

for the chemotherapy studies. In addition, the procurement via purchase of large quantities of certain bulk chemicals and drugs needed by the Program is included in the preparative activities.

The analytical-quality control activities provide the analytical check on the identity, assay and purity of bulk chemicals and formulated drugs used in toxicological, pharmacological and clinical studies. The analytical data required by the Food and Drug Administration (FDA) for the filing of Investigational New Drug (IND) applications is also provided.

II. Natural Products Section: The objective is the development of novel drugs from: 1) higher plants and animal (marine and insect) sources and 2) microbial sources. In support of these tasks, the Section maintains a worldwide program (partly contract and partly non-contract) for the collection of natural products, the preparation of extracts, the isolation and identification of the active components; and in collaboration with the Chemical and Drug Procurement Section, provides for the acquisition of large quantities of active materials needed for toxicological and clincal studies. Also, the Section directs a program for the investigation of three groups of organisms, the bacteria, the fungi, and the actinomycetes. This work requires development and chemical isolation. Surveillance of the literature is maintained as a means of obtaining new materials.

III. Clinical Drug Distribution Section: The objective of this Section is to provide the Program with suitable formulated drugs for clinical evaluation. The principal activities include development of experimental dosage forms, production of various dosage forms for clinical investigative trial, surveillance of the quality of such dosage forms, procurement of commercially available pharmaceutical products and storage and distribution of the clinical products used in the Cancer Treatment Program. In addition, the Section provides the necessary supportive data for the filing of IND applications with the FDA.

IV. Chemical and Drug Information Section: The objective of the Section is to develop data processing systems to meet the scientific, operational control, and management planning needs of the Branch. The Section is primarily concerned with 1) directing contract effort to implement a new automated Chemical Information System (CIS) which supports such activities as accessioning, storage, and distribution of compounds acquired for antitumor testing; 2) directing and coordinating the activities of the contractor responsible for operating the Chemical Information Systems. In addition, the Section is engaged in pursuing a project to improve the utilization of automated literature and chemical files maintained by Federal agencies and industrial organizations to acquire compounds of interest to the program.

The Branch is involved in developing methodology to preselect compounds and materials for the primary screen. Several meetings were held with

resistance to degradation or greater binding to appropriate enzyme sites. Synthetic analogs also may produce longer or shorter duration of activity as the needs dictate, alter the solubility characteristics and improve the stability. Structural modification could also introduce new chromophore groups to aid in determining the drug level in the host. Analogs would also be used in mechanistic studies, and biochemical investigations which include areas of whole body drug distribution, drug metabolites, enzymatic activity, and the route and rate of excretion.

New chemical structures or analogs, developed through biochemical cell cycle kinetic studies, pharmacological and pharmaceutical rationales, and biological activities demonstrated in non-cancer programs, should provide additional input to the directed preselection and synthesis approach. Plans are underway to group together those agents which have shown no cross-resistance and determine if there are any structural similarities for future analog design. The same study will be made on those agents which have shown potentiations in classical biological and/or biochemical screens.

In addition to the search for 'mitotically linked drugs', directed synthesis should also be aimed at affecting other phases of the cell cycle than the synthetic (S) phase or the mitosis (M) phase. The search for agents which have a high degree of organ localization will be undertaken and compounds containing this property should be considered as potential leads for chemical modification. Such specific localizing agents have been encountered with compounds containing the nitroso-moiety which may penetrate the blood-brain-barrier. A program has been developed to synthesize CNS active antitumor agents. Also, drugs localizing in the lung, breast, colon, bladder, etc. will be considered in modifying their chemical structure with the objective of introducing antitumor properties. Organ specific screens are needed to evaluate such new types of agents. The increasing awareness that many antitumor drugs may possess latent carcinogenic properties will be considered in the hopes of eliminating such characteristics. Synthetic development may provide improved methods of synthesis which would achieve economic advantages in the production of large amounts of drugs for the clinic.

The Branch is continuing its search for natural products, which often provides an invaluable source of medicinal agents and new chemical structures. Fermentation products continue to provide many extracts with significant tumor inhibitory activity. It is important that the compound responsible for this activity be isolated and characterized. Some will be useful in chemotherapy, others will be useful as tools in elucidating selective growth regulating mechanism or as templates for syntheses of more effective agents.

Chemical modification and structural analogs of natural products will also provide unique compounds of interest. The continued follow-up of empirical observations and changes in the structure of the already effective agents will also add to our array of new material for investigations. The random but unique structures acquired from natural products higher plants, fermentation products, algae, marine and insect products will continue to be explored.

The Branch is keenly interested in new approaches to drug design. One such approach being exploited is the synthesis of analogs to develop and exploit structure-activity relationships. When a significant new activity has been established, there is need for a major effort to prepare and study related derivatives for the purpose of defining the relation of structure to biological activity. It is desirable to study a series of closely related active compounds to determine toxicity, transport, and other chemo-physical properties. Recognizing that such techniques are still in the early stages of development, effort will continue to improve and develop new methodologies. Several problems do still exist in this area of investigation; such as, obtaining the physical-chemical measurements which are quite exact whereas the biological testing data has a considerable amount of variation. It must be recognized that this approach may not necessarily give immediate answers since it may be necessary to develop new parameters. However, it is hoped that such studies will focus attention on synthetic areas of greatest potential and provide the chemist with more directed synthetic approaches. This is not to be interpreted that new structural classes will be by-passed.

Worldwide Surveillance

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees and academic institutions, research organizations and other governmental laboratories, since a substantial number of compounds are contributed to the Program on a voluntary basis from these sources. Good public relations must be and have been maintained by reporting of data and by making special handling of unusual agents a matter of normal service. With the increasing complexity of data processing and growth of the Program, constant awareness of the problems of 'Commercial Discreet' data must ever be present. A total of 3397 individual suppliers, of which 1397 are industrial suppliers, have contributed samples to the Program. During 1975, there have been 174 new non-industrial suppliers and 89 new industrial suppliers submitting materials for primary screening. Even though the majority of these contributors are domestic suppliers many materials have been supplied from foreign countries. The increasing number of contributors is an index of the continued enthusiasm for the Program by the scientific community.

The Branch's continuous international surveillance for acquiring new agents, synthetic and natural products, has provided over 560 foreign suppliers from industry, academic institutions and governmental and research organizations. Close contact is maintained with these and other potential new suppliers to provide interchange of chemical data and biological information on potential chemicals and drugs of interest to NCI and the scientific community-at-large. International visits were made by the Drug Development Branch staff during this period to the following:

Dr. Wood

Drs. G. Lancini, P. Sensi, Dow/Lepetit, Milan, Italy
Drs. F. Arcamone, L. Bernardi, Farmitalia, Milan, Italy
World Health Organization Conference, Milan Italy

The Branch monitors two miscellaneous contracts, TABLE IV. The Starks C.P., Inc. activities include: 1) selective acquisition of novel chemicals and drugs for the primary screen, 2) serves in a liaison capacity to develop new sources of compounds, 3) prepares the necessary documentation for the computer registration of samples, 4) monitors the flow of computer generated out-puts to suppliers and to management, 5) maintains accurate records of all transactions dealing with liaison activities with suppliers, the operation contractor for the Chemical Information System and with the contractor for storage and distribution of chemicals. The second contract, Microbiological Associates, Inc., provides the Division of Cancer Treatment with services for the storage of bulk chemicals and for the distribution of these materials to research laboratories throughout the world. The contractor also maintains an inventory and shipping record for these bulk materials. Custody and records of the 'Drug Abuse' materials are also maintained.

Accomplishments

The Drug Development Branch provided a steady flow of materials into the Program. These materials consisted of chemicals, plant products, marine and insect products and fermentation products. Some of these materials have demonstrated confirmed activity in one or more of the primary screens (Step III) and have been assigned as candidates for preclinical toxicological evaluation (Decision Point II). Four Investigational New Drug Applications (IND) have been submitted to the Food and Drug Administration (see Table III). Of the 21,295 new crystalline materials added in 1975 to the Program, 1046 compounds (4.9%) were acquired under contract, 719 compounds (3.4%) were purchased, 15,588 compounds (73.2%) were voluntarily submitted from industrial sources, 3,942 compounds (18.5%) were from non-industrial suppliers.

The Branch manages a total of 55 contracts with industrial organizations, academic and research institutions, both domestic and foreign, totaling approximately 13.5 million dollars (FY-76) and covering the major areas as shown under the primary responsibilities of the Branch. Of the 55 contracts, 53 are domestic and 2 are foreign contracts.

In summary, the following were achieved during the calendar year of 1975 by the Drug Development Branch:

1. A total of 31102 new substances (Table I) was obtained for screening or other evaluation;

    a. 20943 synthetics

    b. 1328 antibiotic cultures

    c. 6393 plant materials

    d. 2086 animal materials

    e. 352 other crystalline materials

2. A total of 1287 compounds were prepared under contract;

   a. 241 were resynthesized by the preparation laboratory contracts

   b. 928 were synthesized as new compounds from synthesis and prep lab contracts

   c. 118 were from the natural product laboratory contracts

3. Eighteen new substances were assigned to Decision Network IIA (Table I and II) (candidates for preclinical toxicology);

   a. 3 ferentation products

   b. 1 plant product

   c. 1 animal product

   d. 13 synthetic compounds

4. Four drugs were assigned IND numbers (Table I and III)

   a. 1 fermentation product

   b. 1 plant product

   c. 2 synthetic compounds

5. Seven hundred fifty-nine compounds were assigned to Step III (Table I);

6. Analytical-Quality Control determinations were made on 245 different lots of 91 different compounds representing 147 lots of bulk compounds and 98 lots of clinical formulations;

7. Twenty-eight radiolabeled compounds (Table VI) were procured;

8. Nine new dosage forms were developed and 13 existing dosage forms were modified;

9. Approximately 7,249,461 formulated units were procured;

   a. 5,796,400 compressed tablets and hard gelatin capsules

   b. 1,453,061 vials

10. Fifty-five contracts were supervised by the Branch;

   a. 19 synthesis laboratories

b. 9 preparation laboratories

c. 9 plant and animal products

d. 5 drug formulations

e. 5 fermentation products

f. 2 analytical-quality control

g. 2 radiolabel laboratories

h. 4 miscellaneous

11. There was one Grant Proposal and six Patent Applications reviewed and commented upon;

12. Four patents issued to our contractors;

13. Samples were received from 89 new industrial concerns and 174 new non-industrial sources;

14. Seventy-two manuscripts by contractors were cleared for publication and two manuscripts were published by the Branch staff;

15. Three work sessions and 10 seminars were sponsored.

TABLE I

MATERIALS ACCESSIONED AND ACTIVITY REPORT[1]

	CRUDE EXTRACTS				CRYSTALLINE							
	NO. SUBMITTED		NO. ACTIVE		MATERIAL SUBMITTED		STEP III[2]		DECISION POINT II[3]		IND FILED	
	1974	1975	1974	1975	1974	1975	1974	1975	1974	1975	1974	1975
Fermentation Products	725	1328	174	141	94	159	15	29	4	3	2[4]	1
Animal Products	2011	2086	221	102	76	38	--	8	--	1	--	--
Plant Products	5830	6393	549	346	104	155	10	30	--	1	--	1
Synthetics	--	--	--	--	39540	20943	926	692	3	13	1	2
Total	8566	9807	944	589	39814	21295	951	759	7	18	3	4

(1) Based on Calendar Year 1975

(2) Showing Confirmed Activity

(3) Selected Candidates for Preclinical Toxicological Evaluation and Clinical Trial

(4) Vaccines

TABLE II

COMPOUNDS PASSING DECISION NETWORK IIA

NSC NO.	COMPOUND NAME	SOURCE
5159	Chartreusin, dihydrate	Fermentation
59265	Hematoporphrin	Animal
69587	Metronidazole	Synthetic
95466	Urea, 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitroso	Synthetic
137679	Guanosine, 6-seleno-	Synthetic
169780	2,6-Piperazinedione, 4,4'-(1-methyl-1,2-ethanediyl)bis-, (ICRF-187)	Synthetic
172112	1,3-Diazaspiro[4.5]decane-2,4-dione, 3-[2-[bis(2-chloroethyl)amino]ethyl]- (Spirohydantoin Mustard)	Synthetic
192965	2-Aza-8-germaspiro[4.5]decane-2-propanamine, 8,8-diethyl-N,N-dimethyl-, dihydrochloride	Synthetic
204985	Sterigmatocystin	Fermentation
208642	FILIPIN, a Pentaene	Fermentation
224131	L-Aspartic acid, N-(phosphonoacetyl)-, tetrasodium salt (PALA)	Synthetic
227262	Compound from Bouvardia ternafolia	Plant
246112	Hydrazinecarbothioamide, 2-[(5-amino-4-methyl-1-isoquinolinyl)methylene]-	Synthetic
246131	Adriamycin, N-trifluoroacetyl, pentyl ester (AD-32)	Ferm/Synthetic
247561	Ledacrine	Synthetic
249992	Methanesulfonamide, N-[4-(9-acridinylamino)-3-methoxyphenyl]	Synthetic
261036	1,2-Propanediol, 3-(2-nitro-1H-imidazol-1-yl)-	Synthetic
261037	1H-Imidazole-1-ethanol, α-(methoxymethyl)-2-nitro-	Synthetic

TABLE III

IND'S FILED

NSC NO.	COMPOUND NAME	SOURCE
126771	1,4-Naphtoquinone, 2-(3,3-dichloroallyl)-3-hydroxy	Synthetic
141537	Anguidine	Fermentation
150399E	Spironolactone	Synthetic
153858	Maytansine	Plant

TABLE IV

MISCELLANEOUS BRANCH CONTRACTS

CONTRACTOR	INVESTIGATOR	CONTRACT NUMBER
Microbiological Associates, Inc.	Mozejko	NO1-CM-43793
Starks C. P., Inc.	Paull	NO1-CM-53745

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CHEMICAL AND DRUG PROCUREMENT SECTION

DRUG DEVELOPMENT BRANCH

Scope

The function of the Chemical and Drug Procurement Section is to synthesize new compounds for antitumor evaluation; to procure large quantities for confirmational and other testing, toxicological and pharmacological evaluation, and clinical trial in humans; to procure radiolabeled materials; and to evaluate the materials as to their identity, assay and purity.

The Work of the Section is divided into the following three principal areas:

1. The Synthesis Activities, which provide new compounds for primary screening;

2. The Preparative Activities, which synthesize and isolates (a) bulk chemicals and drugs and (b) radiolabeled materials for toxicological, pharmacological and clinical investigations, for confirmational testing and for completion of primary evaluation; and

3. The Analytical-Quality Control Activities, which provide the analytical check on the identity, assay and purity of compounds and formulated drugs entering the toxicological, pharmacological and clinical programs. Solubility, stability, and cost data are also acquired as is structural analysis.

Staff

The staff of the Chemical and Drug Procurement Section consists of four professional staff and a secretary. One professional person is involved full time with the quality control activities. The professional staff members serve as project officers on the various contract activities of the Section as well as on other contracts that are monitored by the Drug Development Branch.

Synthesis Activities

The objective of the synthesis laboratories is to provide the program with new chemical agents for anticancer evaluations. The laboratories are designed to supply compounds of diverse structures and complexity. The compounds selected for preparation are based upon the following criteria:

1. Modification of clinically active compounds;

2. Biologically active natural occuring compounds;

3. Leads generating from the screening program;

4. Biological and biochemical rationale;

5. Leads from the literature;

6. Compounds to explore structure-activity relationships;

7. New structural types; and

8. Agents to resolve questions on chemical and biochemical mechanism of action.

Even though major advances have been made in recent years, the treatment of cancer is still limited by the availability of useful agents. The usefulness of the majority of the present agents are limited by severe toxic side effects and/or are associated with the development of drug resistant tumors. In addition, many tumors do not respond to any of the current drugs. Therefore, a greater variety of agents with diverse structures and mechanism of action, broader tumor spectrum, and greater safety would be extremely valuable in cancer treatment. The problem of finding these improved drugs is the main objective of the contract synthesis program and its efforts are channeled into many areas of investigation. The following are some examples:

1. A significant amount of the synthetic effort of the Section has been directed toward the synthesis of natural products, their analogs and derivatives. Several products are not available in the quantities necessary for complete evaluation from natural sources and/or are very expensive, and if the structure is known, attempts are made to prepare the material by synthetic means where feasible. Many times the investigation of a synthetic route to the preparation of a natural product affords, or affords the means to, the preparation of analogs which may be more active and interesting than the natural material. It may also be desirable to prepare synthetic analogs and derivatives of naturally occurring material to develop the parameters of an interesting lead or in the hopes of reducing adverse toxicity or metabolism while retaining activity. Recognizing that natural materials are one source of interesting structures and compounds, quite often analogs and derivatives of these can be procured only by synthetic means.

   Some synthetic effort has been and is being devoted to the development of a general and versatile synthesis of the highly complex but clinically effective dimeric Vinca alkaloids, vincristine and vinblastine. The synthesis of related analogs and derivatives is also being undertaken. These analogs and derivatives should provide information concerning the mechanism of action, their metabolism and structure-activity relationships. Also, a synthesis of these materials would provide the means of obtaining radiolabeled vincristine, vinblastine, and related dimeric alkaloids for metabolism and drug distribution studies. The Vinca alkaloids, from the plant Vinca rosea L, are obtained via a very complex and costly extraction procedure. The compounds are present in only minute quantities in the plant. The development of a synthesis for these

materials has the potential of providing the means of obtaining analogs, and of making these materials available in larger quantities and at a lower cost. Considerable progress has been made in the development of the laboratory synthesis of dimeric Vinca alkaloids. In particular, a new approach termed the "biogenetic approach" has been explored in depth and shown to provide an exciting synthetic pathway to novel analogs in the vinblastine family. Catharanthine N-oxide or related N-oxides are coupled with vindoline or vindoline analogs to provide good overall yields of synthetic dimers possessing the correct stereochemistry at C_{18}', the crucial center involved in linking the two "halves" of the dimeric system. The synthetic products are isolated in good yields and a patent application for this method has been filed. This work is being done by the University of British Columbia and the work is continuing.

The clinically effective antibiotics adriamycin and daunorubicin are obtained in rather poor yields from the natural source. These compounds have dose limiting toxicological (cardiac) properties. Some of the synthetic effort of the Section has been devoted to preparing analogs and derivatives of these agents to explore the parameters of structure-activity relationships and in an attempt to obtain compounds with less toxicity. Several semisynthetic analogs and derivatives of these agents have been prepared. These include modifications on both the aglycone portion and the sugar portion of the molecule. The compounds prepared have been submitted for antitumor evaluation and cardiotoxicity studies. This is being done by Stanford Research Institute and Farmitalia (NO1-CM-57014) and it is being continued.

The synthetic effort at Stanford Research Institute directed toward the development of a total synthesis of adriamycin and daunorubicin was completed as part of a cooperative effort with Professor Andrew Kende, University of Rochester. A tetracyclic ketone has been converted to adriamycinone which when coupled with daunosamine gave the desired antibiotic. The tetracyclic ketone intermediate has been prepared synthetically previously. Evaluation of this method to determine its practicality is in progress.

In addition to the above effort related to adriamycin and daunorubicin, some fraudulent aglycones (anthracyclines) and aminosugars are being prepared by Farmitalia (NO1-CM-57036) and Ohio State University, respectively. These fraudulent components will be coupled with natural and fraudulent counterparts. A few of both of these fraudulent components have been prepared and are in the process of being coupled with the natural counterpart. The work in this area is continuing.

Considerable progress has been made by Massachusetts Institute of Technology toward the total synthesis of the clinically effective antibiotic Bleomycin. This complex antibiotic has been divided into five unique fragments whose synthesis is being undertaken separately. Several key

intermediates in the synthesis of three of the five fragments have been prepared and samples of these intermediates have been submitted for screening. The synthesis of a fourth fragment has been achieved and the optimization of the synthetic sequence is in progress.

A systematic study of the positional and structural isomers of the antileukemia alkaloids coralyne and nitidine has been made by Midwest Research Institute utilizing the synthetic procedures developed by this group for the synthesis of these alkaloids. The compounds have been submitted for antitumor evaluation and some structure-activity correlations have been undertaken. These compounds have also been used in the N-O-O triangulation study developed by this group.

The antibiotic Streptonigrin has demonstrated a high degree of antiviral activity in addition to antitumor activity and is obtained in rather poor yields from the natural source. Some effort is being devoted to developing a total synthesis of this material, which will also provide new analogs. The synthesis of the C-D rings has been accomplished by Midwest Research Institute. The A-B ring portion of Streptonigrin has been synthesized previously by this group and the total synthesis of this antibiotic is now in progress.

A limited amount of effort has been devoted to the preparation of colchicine derivatives. Several thiocolchicine derivatives have been prepared by Midwest Research Institute and the preparation of deacetylthiocolchicine glycolamide-O-phosphate is in progress. Some effort is being extended to the preparation of some analogs and derivatives of the interesting pyrrolizidine alkaloid, indicine N-oxide and the biochemically interesting antibiotic, sparsomycin. The synthetic analogs are being prepared in an attempt to obtain more active derivatives and to determine the structural parameters for activity. This work is being done by Microbiological Associates, Inc.

A variety of structural modifications of the ansamycin antibiotic geldanamycin have been prepared and submitted for evaluation, including *in vitro* testing for orcornavirus inhibition. This work was done by the University of Illinois.

2. The area of nucleosides has already provided a number of very interesting compounds with antitumor activity. Cytosine arabinoside and 5-FUDR are nucleosides which have demonstrated clinical activity. However, further chemical studies are needed to provide nucleosides of greater specificity and less toxicity and which are resistant to undesirable enzymatic degradation in the cancer patient.

   A wide variety of structural modifications and derivatives of nucleosides has been prepared by Southern Research Institute and the University of Utah and submitted for evaluation during this report period. These include (a) analogs of the nucleoside antibiotics such as tubercidin, toyocamycin, sangivamycin, formycin, formycin B, and puromycin, (b) tricyclic nucleosides, (c) nucleosides of pyrazolo[3,4-*d*]pyrimidine,

pyrazolo[3,4-b]pyridine, imidazo[4,5-c]pyridine, triazolo[4,5-b]pyridine, and imidazo[4,5-b]pyridine, (d) imidazole nucleosides, (e) "Coformycin type" (5:7 bicyclic) nucleosides, (f) "Carbocyclic sugar" nucleoside derivatives, (g) "carbon-carbon nucleosides", (h) 1-deazapurine nucleosides, (i) 8-azapurine nucleosides, (j) "transition-state" nucleosides, (k) "isonucleosides" where the base is attached at C-2 or C-3 of the sugar, and (l) nucleosides with natural bases and fraudulent sugars, with natural sugars and fraudulent bases, and with fraudulent bases and sugars. The work in this area is continuing.

3. Some of the synthetic effort has been devoted to the preparation of analogs and derivatives of folic acid. A new and improved procedure for the preparation of citrovorum factor (5-formyltetrahydrofolic acid) has been developed by Southern Research Institute and the development of this procedure for the large scale production of this material is in progress. Analogs of aminopterin were prepared in which the glutamic acid moiety was replaced by an aminopimelic acid moiety and the benzoyl-glutamic side chain was replaced by a phenylacetylaspartic acid group. An analog of Methotrexate in which the benzene ring was replaced by a tetramethylene group was also synthesized. Several side chain altered variants of Methotrexate were prepared and the preparation of other folic acid derivatives utilizing the newly developed method is in progress.

4. In reviewing the compounds which have shown activity in the program the largest majority of the compounds have contained nitrogen and many have been nitrogen heterocycles. Some of the synthetic effort is being devoted to the synthesis of new nitrogen heterocycles as potential antitumor agents. The emphasis in this area has been on the preparation of pyrazoline N-oxides and triazepine derivatives. Several compounds of these types have been prepared and submitted for evaluation by the University of Colorado and the University of Rhode Island.

5. In order to extend the discovery of the high degree of antitumor activity in the coordination compound cis-diamminedichloroplatinum which is currently being studied clinically, some additional coordination compounds of platinum have been prepared. The emphasis in this effort has been directed towards preparing complexes with improved water solubility and minimum toxicity. Several complexes have been prepared by the Veterans Administration Hospital of South Carolina substituting cyclohexyldeamine for the ammine groups and the chlorine atoms replaced by solubilizing groups.

6. Some of the synthetic effort of the Section has been directed towards the synthesis of compounds which potentially may localize in specific organs or parts of the body. The main effort in this area during this report period has been to develop CNS active antitumor agents. Some derivatives of the aminoisoquinoline and the aporphine structures have been prepared by Northeastern University and submitted for evaluation.

7. The class of compounds known as N-nitrosoureas have demonstrated a high degree of antitumor activity and some possess the ability to cross the blood-brain barrier. Three compounds of this class (BCNU, CCNU, and Methyl CCNU), are of prime clinical interest. A few additional examples of this structural type have been synthesized by Southern Research Institute with emphasis on mono-and di-hydroxy derivatives of CCNU and analogs of chlorozotocin using other aminosugars.

8. Some effort has been devoted to the synthesis of analogs and metabolites of cyclophosphamide. This clinically effective agent is itself inactive and becomes effective only after appropriate activation in vivo. Aldophosphamide, the key intermediate in the cyclophosphamide metabolism, and a series of analogs of aldophosphamide have been prepared. Additional examples of potentially latent forms of phosphoramide mustard and its analogs have also been synthesized and submitted for testing. The work in this area has been performed by Collaborative Research, Inc., and Southern Research Institute.

9. The design and synthesis of oligo and polynucleotides as potential inhibitors of the RNA dependent DNA polymerases and other polymerases are under investigation. Some oligoribonucleotide primers, inhibitors, and modified templates were prepared by Collaborative Research, Inc.

   In addition, some synthetic effort has been devoted to the synthesis of some derivatives of 3-formylrifamycin as potential polymerase inhibitors. Also, some ansa bridge modified rifamycins have been prepared and submitted for evaluation. Several novel rifamycin derivatives have been prepared as potential inhibitors of focus formation. This work is being done by Dow Chemical Company.

10. Some of the synthetic effort of the Section has been directed towards the synthesis of Procarbazine analogs and related methylhydrazine derivatives. Several compounds of this structural type have been prepared and submitted for evaluation by Midwest Research Institute.

11. A few ethidium, triazolium, and phenanthridinium analogs have been prepared by Microbiological Associates, Inc., and Stanford Research Institute. Compounds of these types have demonstrated activity in the screen and the compounds have been prepared to investigate the parameters of activity.

One very important advantage of synthesis contracts of this type is that they are placed with laboratories which are able to offer a quick response to a new lead and move quickly into an area of research generated by the Cancer Treatment Program and the various clinical groups. The broad experience and mature judgment in synthetic chemistry of these laboratories place them in a unique position for all types of synthesis. These contractors have provided a great number of compounds with a wide variation in chemical structure. During the past year, eighteen new compounds passed Decision Point II, of which thirteen were synthetic materials. These new synthetic compounds are listed in Table II.

The Chemical and Drug Procurement Section directs 19 contracts for the chemical synthesis of new compounds for the Program (Table V).

TABLE V
CONTRACT LABORATORIES FOR SYNTHESIS

Contractor	Investigator	Contract No.
British Columbia, Univ. of	Kutney	NO1-CM-23223
Central Drug Research Inst.	Dhar	PL-480
Collaborative Research, Inc.	Friedman	NO1-CM-60506
Collaborative Research, Inc.	Friedman	NO1-CM-12326
Colorado, University of	Hassner	NO1-CM-43779
Dow Chemical Company	McCarthy	NO1-CM-33724
Farmitalia	Bernardi	NO1-CM-57036
Farmitalia	Arcamone	NO1-CM-57014
Illinois, University of	Rinehart	NO1-CM-23208
Massachusetts Institute of Technology	Hecht	NO1-CM-43712
Microbiological Associates, Inc.	Dubois	NO1-CM-43761
Midwest Research Institute	Cheng	NO1-CM-33743
Northeastern University	Soloway/Neumeyer	NO1-CM-53741
Ohio State University	Horton	NO1-CM-57015
Rhode Island, University of	Goodman/Abushanab	NO1-CM-43778
Southern Research Institute	Montgomery	NO1-CM-43762
Stanford Research Institute	Henry	NO1-CM-33742
Utah, University of	Townsend	NO1-CM-43806
Veterans Administration	Gale	YO1-CM-60102

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories, and are designed and selected to prepare known chemicals and bulk drugs which are needed by the Program. The compounds selected for preparation are not readily available in the quantities needed from the origina supplier or on the open market. These laboratories differ from the synthesis laboratories in that they are service contracts for resynthesis of known chemicals and for the large scale extraction, isolation, and purification of materials from natural sources.

These laboratories are also used to obtain data for the preparation of the necessary quantities of clinically-important chemicals and to develop the most economical means for their preparation. It should be pointed out that many methods of synthesis which are practical for small quantity are not technically feasible or economically practical when used for a large scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies, and cost data are also provided by these contractors. These contractors have, on occasion, provided material in formulated dose form.

The increased emphasis in clinical investigations has caused a major increase of the preparation activities. An increase in the number of compounds demonstrating confirmed activity will result in an increase in the number of compounds to be resynthesized. Also, the increased effort being devoted to toxicological and clinical studies has resulted in the need for even more compounds from this program and in larger quantities.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs.

1. Bulk Chemical and Drugs

The compounds chosen for resynthesis at the preparation laboratories are assigned for resynthesis for five major reasons: (a) clinical investigations; (b) preclinical toxicological and pharmacological evaluation; (c) maintaining a stock of chemicals of clinical and preclinical interest; (d) completion of screening or confirmational testing; and (e) use as intermediates in further synthesis. The compounds so chosen are placed on priority lists based upon their relative importance, with clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from 1 gram to 200 kilograms. Factors governing the amounts depend upon: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the clinician in the quality of the drug he uses.

The Chemical & Drug Procurement Section, through the preparative laboratories, directed the resynthesis of 241 compounds totaling 236 kilograms during the calendar year 1975. Included in this group were 46 compounds scheduled for or undergoing clinical evaluation totaling 226 kilograms. In addition to the compounds resynthesized, 99 new compounds were also prepared by the preparative laboratories. The Section also purchased 20 compounds scheduled for or undergoing clinical evaluation totaling 246 kilograms.

The Section directed nine contract laboratories for the preparation of materials needed by the Program (Table VI).

TABLE VI

CONTRACT LABORATORIES FOR PREPARATION
OF CLINICAL AND EXPERIMENTAL COMPOUNDS

Contractor	Investigator	Contract No.
Aldrich Chemical Company	Li/Wickersham	NO1-CM-23706
Ash Stevens, Inc.	Ash	NO1-CM-57012
Cordova Chemical Company	Jagow	NO1-CM-57031
Dow Chemical Company	Collins	NO1-CM-53768
Monsanto Research Corporation	Ellard	NO1-CM-33709
Parke, Davis & Company	Short	NO1-CM-23708
Pharm-Eco Laboratories, Inc.	Draper	NO1-CM-23707
Starks Associates, Inc.	Starks	NO1-CM-23203
Starks Associates, Inc.	Schultz	NO1-CM-53769

2. Radiolabeled Materials

The Section coordinates the procurement and distribution of radiolabeled chemicals and drugs for pharmacological and drug distribution studies. Materials not available from commercial sources are prepared by contract preparative laboratories. These contracts also handle the storage and distribution and perform the necessary analytical work for all labeled materials, whether prepared under the contracts or acquired from other sources. All requests for radio-active materials are reviewed by a committee in relation to proposed use prior to assignment to the contracts for procurement. A total of 28 radiolabeled materials (Table VII) have been procured from all sources. One hundred and thirty shipments of radio-active substances were made during calendar year 1975.

TABLE VII

RADIOLABELED MATERIALS PROCURED

NSC No.	Radiolabeled Materials	Label
739	Aminopterin	H^3(3',5')
740	Methotrexate	H^3(3',5')
752	6-Thioguanine	C^{14} (8)
8806	Melphalan	C^{14} (Ring)
8806	Melphalan	C^{14} (Ethylene)

NSC No.	Radiolabeled Materials	Label
13875	Hexamethylmelamine	C^{14} (2,4,6-methyl)
19893	Uracil, 5-fluoro-	C^{14} (2)
26271	Cyclophosphamide	C^{14} (Ring-C_4)
63878	Cytosine, 1-β-D-arabinofuranosyl- (Ara-C)	H^3
63878	Cytosine, 1-β-D-arabinofuranosyl-	C^{14} (2)
77213	Procarbazine	C^{14} (N-methyl)
79037	Urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso- (CCNU)	C^{14} (cyclohexyl-C_1)
82151	Daunomycin	C^{14} (14)
95441	Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso- (Methyl CCNU)	C^{14} (Methyl)
104800	Galactitol, 1,6-dibromo-1,6-dideoxy-, D-	C^{14} (1,6)
104801	Acrylic acid, 3-(p-anisoyl)-3-bromo-sodium salt (Cytembena)	C^{14} (Ring)
112907	Tetrahydrouridine	C^{14} (2)
118994	Diglycoaldehyde, α-(hydroxymethyl)-α'-(6-hydroxy-9H-purin-9-yl)-	C^{14} (8)
126849	3-Deazauridine	C^{14} (Base-C_6)
126849	3-Deazauridine	C^{14} (Sugar-C_1)
139105	Ethanesulfonic acid, compd. with α-[2-chloro-4-(4,6-diamino-2,2-dimethyl-s-triazin-1(2H)-yl)phenoxy]-N,N-dimethyl-m-toluamide (1:1)	C^{14} (4,6)
148958	Ftorafur	C^{14} (Base-C_2)
148958	Ftorafur	C^{14} (2',5')
154020	1,4,5,6,8-Pentazaacenaphthylene, 3-amino-1,5-dihydro-5-methyl-1-β-D-ribofuranosyl-	C^{14} (N-methyl)
176319	Quinolinium, 6-amino-1-methyl-4-[[[[[[(1-methylpyridinium-4-yl)amino]-phenyl]amino]carbonyl]phenyl]amino]-, dibromide	C^{14} (Carbonyl)

NSC No.	Radiolabeled Materials	Label
178248	D-Glucopyranose, 2 [[[(2-chloroethyl)-nitrosoamino]carbonyl]amino]-2-deoxy-(Chlorozotocin)	C^{14} (Ethylene)
178248	D-Glucopyranose, 2 [[[(2-chloroethyl)-nitrosoamino]carbonyl]amino]-2-deoxy-(Chlorozotocin)	C^{14} (Sugar-C_1)
409962	Urea, 1,3-bis(2-chloroethyl)-1-nitroso (BCNU)	C^{14} (Ethylene)

The Section directed two contracts devoted to the procurement of radiolabeled materials (Table VIII).

TABLE VIII

CONTRACT LABORATORIES FOR

PREPARATION OF RADIOLABELED MATERIALS

Contractor	Investigator	Contract No.
M		
Monsanto Research Corporation	Yanko	NO1-CM-23715
Stanford Research Institute	Leaffer	NO1-CM-12162

Analytical-Quality Control

The Branch is keenly aware of the need for providing bulk chemicals and formulated drugs of highest quality for use in clinical investigations and preclinical evaluations. The purpose of quality control, based on rigorous chemical analytical methods, is to evaluate these materials as to their identity, assay, and purity. All lots of bulk materials to be used in toxicological and pharmacological or in clinical studies are sent for analysis before the materials are released for use or formulation. Also, quality control data is obtained for all formulated materials prior to their release to the clinical investigators.

It should be pointed out that the Cancer Treatment Program deals with a great diversity of chemical compounds, many of which are entirely new with no methods of assay available. Very few compounds are assayed on a routine basis; thus, in many cases, methods of assay must be developed. These assay methods are made available and serve as an invaluable tool to the investigator using the particular compound. Also, the program on analytical studies of anticancer compounds has become involved in working out analytical methods for many of the compounds in biological media such as blood and urine.

Among the techniques that are most commonly used in the drug analysis work are: spectroscopic determinations (ultraviolet, visible, infrared and nuclear magnetic resonance), chromatographic determinations (paper, thin layer, high pressure liquid, and gas-liquid) and titrimetric determinations (aqueous and potentiometric). In addition to the above, elemental microanalysis is carried out. Where applicable, optical rotation, optical rotatory dispersions, refractive index, functional group assay, the degree of hydration or solvolysis determinations and the preparation of derivatives are performed. Chromatographic procedures are most useful in the determination of the presence of a possible unidentified impurity. The combination of this procedure with the melting point, infrared and ultraviolet spectra is often sufficient for the analysis of a simple, stable, well-characterized compound. The determination of the purity of a more complex compound is often an analytical research problem requiring the use of a combination of sophisticated techniques and the consideration of the stability of the compound and its possible contaminants or decomposition products.

In addition, the analytical unit is concerned with those physical and chemical characteristics of the compounds which must be considered in the handling, storage, formulation, and administration of the material. Of increasing importance to the Program is solubility and stability data, which serves as a guide for drug formulation, storage, and shipping. The analytical data on compounds of current interest is periodically reviewed and the data is assembled in the form of a Chemical Information Sheet. These Chemical Information Sheets are made available to the investigators using the material.

The need for the evaluation of the "Shelf-life" of formulated drugs is an increasing problem. In order to establish the effective life of a drug, the continuing assay of these materials is required and is performed on a regular basis.

In addition, the necessary analytical data for the Food and Drug Administration are collected and assembled. Files are maintained on the drugs in clinical trial in compliance with the Food and Drug Administration regulations covering "Investigational New Drug Applications."

The Unit collects and files such data as:

1. The detailed method of preparation;
2. The supplier's analytical sheet; and
3. The independent analytical evaluation of each lot of chemical or drug as evaluated by the analytical-quality control contracts.

The assembled data is of use in writing purchase specifications. Attempts are made to keep specifications brief by listing those requirements which are judged to best identify and assay the compounds.

Another function of the quality control group is the analytical evaluation and/or certification of materials purchased on the open market. Inquiries received from scientific investigators and participating clinicians as to drug quality are also handled.

During the calendar year 1975, there were 211 analytical reports on 91 differe compounds, representing 147 lots of bulk chemicals, and 98 lot of clinical formulations. Each lot is treated individually, and the extent of analysis for each sample is a matter that is jointly decided by the Drug Development Branch and the contractors. The analytical data occasionally indicated unacceptable materials resulting in their being returned to the manufacturer for reprocessing.

Midwest Research Institute, determines the partition coefficient of compounds for use in structure-activity correlation studies. This often involves intensive analytical development in cases where the compound is relatively insoluble or highly soluble in one of the solvents.

Assistance was also extended to the formulation facilities and the preparation laboratories with their analytical and formulation problems concerning compoun of interest to the Program.

The Section directed two analytical-quality control contract laboratories (Table IX). In addition to the two analytical laboratories, elemental analyses are obtained from Micro-Analysis, Inc., under a purchase order agreement.

TABLE IX

CONTRACT LABORATORIES FOR ANALYTICAL-QUALITY CONTROL

Contractor	Investigator	Contract No.
Midwest Research Institute	Haggerty	N01-CM-33722
Stanford Research Institute	Lim	N01-CM-33723

CLINICAL DRUG DISTRIBUTION SECTION

DRUG DEVELOPMENT BRANCH

Scope

The Clinical Drug Distribution Section is primarily responsible for the following major functions:

1. Development of investigational formulations for clinical use;
2. Production and packaging of investigational drugs;
3. Procurement by direct purchase of formulations for clinical use;
4. Quality control surveillance of all clinical dosage forms;
5. Preparation of Pharmaceutical Data Sheets for clinical brochures, IND's, etc.; and,
6. Storage and distribution of clinical dosage forms.

Staff

The staffing of this Section consists of three Public Health Service Pharmacists, one Ph.D. Visiting Fellow, two shipping and receiving clerks, one laboratory technician and one secretary.

Development of New Dosage Forms for Clinical Use

Formulation development commences when an agent passes Decision Network IIA. The first approach to development involves an evaluation of existing chemical and physical data and a review of *in vivo* route and regimen studies in rodents. The accumulation and evaluation of these data are critical to the ultimate design of the dosage.

Developmental studies are assigned to one of the contract pharmaceutical laboratories. In preparing the assignment, the contractor is provided with the route of administration, dose level, and available supporting chemical data. Consultation with the Project Officer is carried out concerning feasible formulation approaches.

Routinely, agents considered for parenteral formulation are subjected to solubility and stability studies in a selected group of physiologically accepted vehicles. The drug solutions are then studied for the effects of pH, temperature, light and atmospheric oxygen. Additionallly, compatibility tests are performed with buffers, preservatives and vial closures. Agents considered for oral formulation are studied for compression properties, compatibility with binders, photodegradation and temperature effects.

Some of the drugs recommended for development are either insoluble or poorly soluble in most commonly used physiological vehicles. Occasionally, these compounds are highly reactive and require special handling. These problems have been an obstacle in the preparation of some parenteral dosage forms of potential antitumor agents and have been explored under a contract with the University of Kansas or in the in-house formulation laboratory.

The University of Kansas has been studying complexation and "pro-drugs" as approaches for parenterally administered antitumor agents. A parenteral formulation of hexamethylmelamine has been developed by formation of a soluble complex with gentisic acid. A combination of complexation and pro-drug techniques have been combined to provide a marked improvement in acronycin solubility. The formulation laboratory has completed a definitive evaluation of process variables on the adriamycin-DNA complex. Since DNA was principally affected, these studies may be applicable to other complexes of intercalating agents with DNA. The vacuum drying process was thoroughly evaluated during the past year. This method is useful in the processing of ethanol soluble materials and may be applicable to a variety of compounds particularly plant products.

Experimental formulations are submitted for evaluation and confirmation of antitumor activity. Simultaneously, with these studies, the dosage forms are sent to independent laboratories for chemical and/or biological analyses.

The toxicological results and the accumulated pharmaceutical development data serve as a basis for determining the starting human dose and the ultimate dosage form to be used in the initial clinical studies. Several dosage strengths of the same investigational drug may be prepared provided the drug concentration remains within the development limits of the dosage form.

During 1975, 25 projects for dosage form development were assigned. Eleven of these involved new drug research and nine assignments were for modification of existing preparations. A total of 22 new dosage forms (IND filings) entered clinical trial from the contract efforts, nine of which were original IND submissions.

Production and Packaging of Investigational Drugs

An important function of the Section is to provide the Clinical Program with sufficient quantities of formulated materials. The production of dosage forms is mainly carried out by contract agreement with qualified pharmaceutical manufacturers.

The production output of clinical dosage forms for 1975 remained at a high level of effort. The formulation contractors produced a combined total of 40,149 bottles of tablets or capsules (2,341,860 oral doses) and 847,000 vials (parenteral doses). Production output which is dictated by clinical needs has continued to increase during the last few years. The production contractors have been able to meet the Program's needs for formulated materials.

Four production contracts and one development contract (University of Kansas) (Table XII) were monitored:

TABLE XII
CONTRACTS MONITORED BY THE CLINICAL DRUG DISTRIBUTION SECTION

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Ben Venue Laboratories, Inc.	Wickes	NO1-CM-53811
Iowa, University of	Wurster	NO1-CM-43743
Kansas, University of	Higuchi	NO1-CM-23217
Philips Roxane Laboratories	Marine	NO1-CM-12139
Tennessee, University of	Avis	NO1-CM-02084

Some contractors primarily engaged in bulk drug preparation have capabilities for dosage form production. The Section encourages these contractors to develop and produce their own dosage forms. Specifications for formulation and production are provided to each contractor. This arrangement has proven economical, expedient and extremely beneficial to the program.

Dosage forms are developed and produced by pharmaceutical manufacturers in cooperation with the Division of Cancer Treatment. These sources include several foreign drug manufacturers. The dosage forms are prepared from specifications which are established by mutual agreement between the Section and the manufacturer. During 1975, the following companies supplied a total of 56,821 injectables and 1,437 bottles of tablets and/or capsules through this joint research effort:

Bristol Laboratories, USA	NSC-157365	Neocarzinostatin
Eli Lilly & Company, USA	NSC-403169	Acronycin
Farmitalia, ITALY	NSC-123127	Adriamycin
Ives Laboratories, USA	NSC-82151	Daunorubicin
Mead Johnson & Company, USA	NSC-109724	Ifosphamide
Sandoz, USA	NSC-122819	VM-26
Sandoz, USA	NSC-141540	VP16-213
Wellcome Foundation, ENGLAND	NSC-220537	Corynebacterium Parvum
Winthrop Laboratories, USA	NSC-142982	Etrenol
E. R. Squibb & Sons, USA	NSC-32065	Hydroxyurea
The Upjohn Company, USA	NSC-85998	Streptozocin

Purchase of Dosage Forms

Another activity is the purchase of formulated products. These items fall into two categories: (1) investigational products and (2) commercial products. The investigational products are formulated proprietary drugs not available for commercial sale, but of interest to the Cancer Treatment Program.

Such products are usually manufactured according to NCI specifications and sold to NCI on a cost reimbursement basis. Commercial products are items available for open purchase. Procurement of all purchased dosage forms is handled through the NIH Procurement Branch. Several procurement mechanisms are used depending on such factors as the type of product, the size of the order and the priority for clinical need.

A total of 386,902 vials and 33,720 bottles of tablets and/or capsules (3,343,500 oral doses) of commercial and investigational dosage forms were purchased during calendar year 1975. All suppliers are required to provide a certificate of analysis on each lot of drug purchased. The data are reviewed prior to release for clinical use.

Table XIII summarizes the procurement activities of the Section during calendar year 1975.

TABLE XIII
DRUG ITEMS PROCURED

SOURCE	Capsules/Tablets 1974	Capsules/Tablets 1975	Injectables 1974	Injectables 1975
Produced by Contractors	1,185,510	2,341,860	677,708	847,000
Contributed or Cost Sharing	600,800	111,080	46,726	56,821
Purchased Invest. Drugs	30,000	45,000	689,480	162,338
Purchased Commercial Drugs	3,744,650	3,298,500	579,599	386,902
	5,560,960	5,796,440	1,993,513	1,453,061

Quality Control of Clinical Dosage Forms

Each lot of formulated drug must meet specific quality control criteria prior to release for clinical use. This Section, in collaboration with the Chemical and Drug Procurement Section, maintains a strict quality control program designed to insure that each batch of formulated material meets the criteria for strength, quality and purity. Parenteral products are tested for potency, sterility, safety, pyrogenicity, etc., and oral products are tested for content uniformity, weight variation, hardness, disintegration, etc. All products are tested for potency by the formulator and sometimes by another laboratory.

Individual unit assays are required; the number of samples assayed depend on the dosage form and the size of the production batch. All new dosage forms are routinely submitted to an outside analytical contractor to verify the analytical methodology and accuracy of the formulator. The confirmation analyses are mainly performed by the contract analytical laboratories, Stanford Research Institute and Midwest Research Institute.

This staff participates in evaluating the quality of chemicals and drugs. The analytical results of bulk chemicals and formulated clinical dosage forms are reviewed. During calendar year 1975, 340 lots of clinical dosage forms were reviewed.

The pharmaceutical contractors are requested to perform compatibility and stability studies on reconstituted and diluted solutions of the investigational dosage form. These studies are carried out under simulated clinical conditions to test the effects of temperature, light and additives.

The accumulated information is then reported on a Pharmaceutical Data Sheet which is incorporated into the Clinical Drug Brochure.

This Section also maintains a shelf life surveillance program on all lots of non-commercial formulated dosage forms. Representative samples of each formulated lot are stored under specified controlled conditions and assayed for chemical and/or physical change. Three assay schedules are used for shelf life studies: Schedule A, designed to provide stability data under accelerated conditions; Schedule B, designed for controlled studies of repeat formulations; Schedule C is assigned to products that have been prepared more than three times. The Schedule C samples are held, but not assayed unless specifically requested. All Schedules provide a minimum four year surveillance record.

Preparation of Pharmaceutical Data Sheets

This Section prepares the Pharmaceutical Data Sheets for all investigational dosage forms used in the Program. The Data Sheets are used in preparing Clinical Brochures and for product information to health care practitioners. The Sheets provide a description of the product including structure and molecular weight and also provide reconstitution information as well as stability data. This Section has collaborated with the Investigational Drug Branch, Cancer Therapy Evaluation Program, for the publication of these Data Sheets.

The Section also prepares Investigational New Drug (IND) attachments I, II, III, V and VII for submission to the Investigational Drug Branch for FDA-IND filing. These attachments consist of manufacture, control and packaging data required for investigational dosage forms.

The Section has been instrumental in the formation of a Special Interest Group (SIG) dealing with the problems of Oncology through the American Society of H spital Pharmacy. The Section was responsible for the presentation of a program dealing with the pharmaceutical aspects of the treatment and management of cancer patients which resulted in the formation of the aforementioned SIG.

Storage and Distribution of Clinical Dosage Forms

The receiving and shipping activities are carried out by two shipping clerks. These clerks have been instructed in the required handling techniques for each drug. They are knowledgeable of postal and freight procedures used in this country and throughout the world. A significant amount of clinical drugs are shipped to foreign investigators each year. During 1975, 1,172 foreign shipments were made. During the same period, 10,021 domestic drug shipments were made.

During 1975, a total of 1,432,261 drug items (vials and bottles) were received and inventoried for subsequent worldwide distribution. During that same period, 1,395,366 drug items were distributed, averaging 45 individual shipments daily.

Another phase of the Clinical Drug Distribution program involves the management of returned drugs from clinical investigators. Returned drug information is recorded on the original inventory file, including the name of the investigator, the amount of drug returned and the date received. This information completes the distribution cycle of the drug since the original shipping date is also on file by investigator's name, quantity shipped, etc. This Section is responsible for the disposition of the returned drug. The judgement to restock, destroy or quarantine a returned drug is dependent upon the condition of the drug container, the age and known stability of the dosage form, the amount of drug in question and other factors.

During 1975, an average inventory of 112 dosage forms by lot number involving 180 different formulations were handled. These figures include investigational and commercial dosage forms.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03584-04 DDB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Research in the Dosage Form Development of New Antitumor Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Davignon	Head, Clin. Drug Dist. Sect.	DDB NCI
OTHER:	A. Rahman	Visiting Fellow	DDB NCI
	J. C. Cradock	Pharmacist	DDB NCI
	H. B. Wood, Jr.	Chief, Drug Dev. Br.	DDB NCI
	S. Takahashi	Biologist	DDB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Drug Development Branch

SECTION
Clinical Drug Distribution Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

SUMMARY OF WORK (200 words or less - underline keywords)

This project describes the intramural formulation activities with potential anti-neoplastic agents. Formulation problems particularly of a solubility nature are investigated. A tablet formulation of ellipticine, a plant alkaloi with antitumor activity, was developed utilizing its strong complexation with polyvinylpyrrilidone (PVP). Mixtures of ellipticine with PVP of varying molecular weight provided a solubility increase of about 1,000 fold and excellent dissolution characteristics. Physiological disposition and anti-tumor studies in mice indicate good absorption. The effect of a variety of formulation variables on DNA-Adriamycin were evaluated. The quality of the complex was affected by autoclave time, rate of cooling and electrolyte concentration. Low temperature vacuum drying was thoroughly evaluated as a formulation process. A number of compounds were identified as suitable excipients including urea, polyethylene glycol 4000, citric acid, tromethamine and methyl paraben.

Project Description:

General Objectives:

1. Study new approaches with potential utility for those compounds posing difficult formulation problems.

2. Evaluate dosage form problems presented by clinical and laboratory investigators.

Specific Objectives:

1. Formulation of an oral dosage form of NSC-71795 (Ellipticine).

2. Evaluation of processing variables on the efficacy of a DNA-Adriamycin Complex.

3. Evaluation of pharmaceutical additives in the low temperature vacuum drying method.

4. Pharmaceutical properties of intrathecally administered drugs.

Major Findings:

1. NSC-71795 (Ellipticine)

This plant alkaloid demonstrated activity by several routes of administration in experimental tumor systems. Since intravenously administered ellipticine produced hemolysis and hypotension in the dog and monkey, efforts were directed toward an oral formulation. Polyvinylpyrrolidone (PVP) was superior to polyethylene glycol and a variety of surfactants in increasing ellipticine solubility. The molecular weight of PVP influenced ellipticine solubility and the optimal drug concentration (approximately 13 mg/ml) was achieved with PVP 10,000. A number of tablet formulations were evaluated. Dissolution of ellipticine from PVP tablets in water and simulated gastric juice was superior to ellipticine HCl. Rate and maximum dissolution could be affected by PVP mixtures of various molecular weights, thus providing a means for controlling drug release. The diminished dissolution of ellipticine HCl tablets particularly in gastric juice was due to the additional chloride ion in the dissolution medium.

Tissue levels and antitumor activity following oral administration to mice of four formulations were evaluated. The tissue levels of ellipticine in PVP 10,000 or the PVP mixture yielding optimal dissolution characteristics were equal or greater than obtained with ellipticine HCl and twofold to fourfold higher than ellipticine in suspension. Maximum levels usually occurred at eight hours and were highest in liver and

spleen followed by kidney, heart and blood. The antitumor effects of the optimally absorbed preparations (ellipticine-PVP or ellipticine HCl) following p.o. administration were very similar in the i.p. L_{1210} system. The dissolution, distribution and antitumor data indicate that the ellipticine-PVP tablet should be further evaluated in larger animals.

The mechanism of PVP enhancement of ellipticine solubility was evaluated. Solubility, equilibrium dialysis and intrinsic viscosity data indicate that solubilization is due to complexation.

2. DNA-Adriamycin Complexes

In the past few years, a variety of drug-polymer complexes have been evaluated as a means of altering physiologic distribution of antitumor agents thus enhancing efficacy and/or diminishing toxicity. The work of deDuve, Trouet and associates with DNA complexes of adriamycin or daunorubicin has been most extensively studied. Preparation of this product for clinical use requires autoclaving. Since this procedure resulted in partial degradation of DNA, the affect of a variety of formulation processes on the complex needed to be evaluated to insure consistent biologic response. Intrinsic viscosity determinations using a suspended level instrument provided a sensitive indicator of product quality. Intrinsic viscosity was affected by autoclave time, rate of cooling, electrolyte concentration, DNA type and addition of adriamycin, but not lyophilization. Optimal conditions were found to include 15 minute autoclave cycle, slow cooling and a sodium chloride content of 0.1-0.15 M. Spectral properties of the drug-DNA complex were evaluated. However, this method was inferior to viscosity in detecting subtle changes in DNA.

Conditions for large-scale production were also defined. Physiologic distribution, antitumor and cardiotoxicity studies are ongoing.

3. Low Temperature Vacuum Drying

Low temperature vacuum drying is an adaptation of freeze drying that provides a method for sterile preparation of dry solid parenteral formulations from certain organic solvents. As in conventional freeze drying, excipients may be required to provide additional powder mass, increase stability by pH adjustment or retarding oxidation, as well as, protecting against accidental microbial contamination during handling. A wide variety of pharmaceutical excipients were initially evaluated in this system. Urea and polyethylene glycol 4000 were found to be suitable bulking agents in view of their ethanol solubility and drying characteristics. Adjustment of pH can be achieved by inclusion of citric acid, tromethamine or sodium acetate. The parabens are antibacterial preservatives that may be processed in this manner. These

formulation components exhibited required solubility in concentrated and dilute (approximately 5-10%) ethanol, as well as, adequate drying properties. A combination of vehicle components is under evaluation as a means of improving an unstable formulation. This approach may have wider applicability to potent non-alkaloidal plant products that are ethanol soluble.

4. Design of Dosage Forms for Intrathecal Use

From a formulation viewpoint, the intrathecal (i.t.) route has been considered as a subcategory of the intravenous (i.v.) route. However, the rapid dilution and buffer capacity provided by the blood supply may allow wider latitude in terms of pH, electrolyte composition and formulation components than are permissible for intrathecal use. Recently, several reports have indicated that pharmaceutical composition of vehicles may influence toxicity of intrathecally administered anti-neoplastic agents. Therefore, the pharmaceutical properties of methotrexate, cytarabine and hydrocortisone sodium succinate were systematically evaluated in three commonly used vehicles; Sodium Chloride Injection, U.S.P., Lactated Ringer's Injection, U.S.P. and Elliott's B Solution. The choice of vehicle did not affect the chemical or physical stability of the antitumor agents. However, the osmolarity and pH of the Elliott's B Solution most closely approximate cerebrospinal fluid. The osmolarity of Lactated Ringer's is similar to Elliott's B, but the buffer capacity is less. In contrast to the other vehicles, the use of Elliott's B Solution as a diluent for intrathecal use is under IND status.

About six other projects were investigated during the past year. Routine formulation development studies were carried out with nitidine chloride and bruceantin. Several other projects involved cooperative evaluations of formulated materials for pharmacologic and/or toxicologic effects.

In December 1975, a presentation was made at the American Society of Hospital Pharmacists Clinical Mid Year Meeting concerning "Dosage Form Development Studies of Parenterally Administered Antitumor Agents" (J. Cradock). The studies on the Adriamycin-DNA Complex were described at the Parenteral Drug Association's Midwest Meeting in June 1976 (A. Rahman).

Publications: None.

NATURAL PRODUCTS SECTION
DRUG DEVELOPMENT BRANCH

Scope

This Section has as its primary objective the development of novel cancer chemotherapeutic agents from higher plants, animals, and microbial sources. This area will be discussed under the headings: Plant and Animal Products; and Fermentation Products.

Staff

The Section has operated with a staff of four persons, consisting of two professionals (1 Ph.D. and 1 MS) and two clerical employees. Dr. John Douros was appointed Head of the Section.

Surveillance

World-wide surveillance of the published literature and collection of chemical and drugs from natural sources for biological evaluation are carried out in the Natural Products area. An informal literature survey has been conducted which has yielded several interesting compounds to the program such as aclacinomycin, succinylneocarzinostatin, etc.

Accomplishments

The Natural Products Section continues to contribute an increasing number of compounds to the program, many of them of novel structure. Those with the highest activity will be submitted for confirmation and testing in the slow-growing tumors, B16 melanoma and Lewis Lung carcinoma and others such as mouse breast, colon, etc.

Table I lists the materials of natural origin acquired during the year under review compared with the previous year. Of the crude extracts, contractors provided 96% of the 1,328 fermentation products, 50% of the 2,086 animal products, and 90% of the 6,393 plant products. A total of 352 purified natural products were received or isolated. Of those for which results have been obtained, 73 were active and 67 passed the criteria for confirmed activit (Step III). Three fermentation, one animal, and one plant compound were assigned to Decision Network. One plant and two fermentation compounds were assigned IND numbers respectively: Maytansine (NSC-153858), Anguidine (NSC-141537), and Neocarzinostatin (NSC-157365). Fewer plants are being collected for initial extraction while a concentrated effort is being made to obtain reconfirmed active plants in 200-500 lb. quantities in order to expedite fractionation and isolation of the active compounds. The fermentatic program still shows that an *in vitro* cell cytotoxicity pre-screen allows for a much higher yield of *in vivo* actives. In the case of the contractors using a pre-screen, an average of approximately 33% *in vivo* actives was obtained.

PLANT AND ANIMAL PRODUCTS PROGRAM

In carrying out its objectives in the plant and animal products area, the Section has, in addition to the contract effort, developed programs with collaborating research grantees and other organizations in (a) plant and animal collecting; (b) preparation of extracts; (c) fractionation of active extracts, and the isolation and identifications of the active agents; and (d) in collaboration with the Chemical and Drug Procurement Section, the preparation of large amounts of active agents from plant materials for preclinical toxicology and clinical trial.

Through extensive correspondence, contact is maintained with potential domestic and foreign suppliers in order to acquire plant, animal and fermentation compounds which are of special interest to the Program. In addition, this correspondence provides information relating to the use of plants and plant products in medicine (popular and folklore).

The following contractors are engaged in the Plant and Animal Products Program (Table X).

TABLE X

PLANT AND ANIMAL PRODUCTS CONTRACTORS

CONTRACTORS	INVESTIGATOR	CONTRACT NO.
Agriculture, U.S. Department of	Perdue	YO1-CM-40001 (transfer)
Arizona, University of	Cole	NO1-CM-33750
Arizona State University	Pettit	NO1-CM-12308
Hawaii, University of	Sagawa	NO1-CM-33747
Illinois, University of	Farnsworth	NO1-CM-22078
Research Triangle Inst.	Wall	NO1-CM-92019
Virginia, University of	Kupchan	NO1-CM-12099
WARF Institute, Inc.	Coon	NO1-CM-43707

In addition, a contract for the procurement of plant extracts has been continued with the University of Costa Rica (Dr. J. Saenz R.), a similar one for the procurement of marine animal extracts has been continued with the University of Oklahoma (Dr. A. J. Weinheimer), and a marine species procurement (large amounts) with Dr. R. E. Schroeder. Besides the contractors listed above, several groups, including grantees and those not supported by the NIH, contribute in different ways to this program.

Progress was made in the following areas of plant, animal, and fermentation products:

A. Plants

1. Nomenclature Review. Good progress has been made by the U.S.D.A. in reviewing the 81,000-odd botanical names in our plant accessions to assure technically correct Latin names. This will (1) eliminate synonymy thereby avoiding future conflicts where the same plant under two differen names is being fractionated by two different chemists, (2) standardize th nomenclature, and (3) provide accurate nomenclature for future publicatio of plant contractors. The U.S.D.A. is coordinating with NCI's Automated Information Section to rectify any variations in plant nomenclature.

2. Plant Program Monitoring. This is a continuing joint project of the U.S.D.A. and the Section to review the total plant input and the chemical output of the program for the purpose of recognizing plant families that (a) are known to produce compounds of interest to our program or that hav been inadequately screened, in either case making them deserving of special effort in collecting, and (b) are known not to produce compounds of interest to our program, or that have been adequately screened, in either case making them subject to avoidance in collecting.

This selective collection has been implemented for some time and is yielding promising results. Plants which belong to families which have produced compounds of interest are being collected in large quantities suitable for extraction without the necessity for recollection, and already the indications are that a substantially higher yield of confirmed actives is being obtained although in some cases the active compounds are similar. A planned program to collect in countries known phytogeographically to be rich in such families is being pursued.

Thus, by this means, a rational and an empirical approach to plant collecting has been emphasized, combining guidance provided by chemical feedback with modified emploratory collecting necessary to locate new areas.

3. Duplicate Searching. Following the establishment of a computer "terminal" at the U.S.D.A., it is possible for the U.S.D.A. to communicat telephonically with our plant genus and species "bank" and instantly determine duplicates among accessions. Duplicates would be reviewed to determine if they should be evaluated. This procedure could make a great savings in the expense of extracting and screening these plants.

4. Large Recollections. No large recollections (3,000 lbs. or greater) were made this year, although 74 plants were recollected in amounts greater than 100 lbs. Three plants, Jatropha macrorrhiza (875 lbs.), Sesbani drummondii (1,000 lbs.), and Sehrebera cilata (1,185 lbs.) were collected in what is relatively large amounts.

5. Plant Statistics. Sixty-six new genera and 153 new species were extracted this year. Three hundred forty-six extracts were active in PS or KB. One hundred ninty-six PS and one hundred fifty KB extracts have confirmed activity. One hundred fifty five new crystalline plant products were obtained and of these, approximately 75% were from contracts.

B. Marine Organisms

During 1975, 2,086 animal products, mostly marine, were accessioned. Thirty-eight crystalline materials were obtained from this work and are in various stages of testing. Approximately 85% of these were obtained from our contract. Forty-three new genera and sixty-seven new species were fractionated. Large collections of 18 active (T/C>175) marine animals were accomplished and these materials submitted to chemists for extraction, purification and structural elucidation of the active chemical. Fifty-four additional new large marine collections will be made in order to obtain active compounds in enough quantity for evaluation and to determine the productivity of this area of research in obtaining novel antineoplastic agents. Isolation of antineoplastic agents from insects was not rewarding and this program has been phased out.

FERMENTATION PRODUCTS PROGRAM

Antibiotics have a great potential in cancer chemotherapy as primary treatment agents, in combination or as the initial structural building blocks for new and novel compounds that can be synthesized and used in the clinic.

The present fermentation program has been carried out through contracts at different biological laboratories listed in Table XI:

TABLE XI

FERMENTATION CONTRACTORS

CONTRACTORS	INVESTIGATOR	CONTRACT NO.
Bristol Laboratories	Claridge	NO1-CM-43759
Georgetown University	Katz	NO1-CM-43732
Litton Bionetics (Project 17)	Langlykke	NO1-CO-25423*
Michigan Dept. of Health	Olson	NO1-CM-33702
Parke-Davis & Co.	Dion	NO1-CM-43754
Upjohn Co.	Neil	NO1-CM-43753
Microbial Chemistry Research Foundation	Umezawa	NO1-CM-57009

* One of 20 projects under the Litton contract.

A computer analysis of the activity in PS of all the microbial cultures teste in our program revealed seventy-nine extremely active fermentations.

Sixty-six cultures are under chemical fractionation by either the fermentatio contractors or the contract plant fractionators, and it is expected that some will be purified and evaluated in the coming year. Several fermentations hav been assigned to plant fractionators for isolation of the active agents in order to reduce a slight backlog with the fermentation contractors and to tak advantage of the expertise of the plant fractionators. Already carminomycin has been isolated by our plant fractionators from an antibiotic fermentation. Additional antibiotics will continually be obtained from our surveillance program and will be evaluated. A total of 159 crystalline microbial metabolites were tested with 90% coming from surveillance.

Project 17. A fermentation project has been initiated at the Frederick Cance Research Center to develop an in house fermentation program.

The pilot plant program is to be used to produce large quantities of antineoplastic antibiotics which have been obtained from our present contracts or in hourse research at FCRC. The pilot plant will obtain engineering, microbiological, and chemical data on the various processes and do the necessary developmental research to improve and decrease costs of the antineoplastic agent. Limited pilot plant capacity has been a major drawback in our present contract fermentation program. Materials needed for chemical purification, advanced testing, etc. have not been more rapidly forthcoming because of direct competition for facilities with the pharmaceutical companie own research program. The pilot plant facility will be completed by March 25, 1976. Several enzymes have been prepared in 30 gallon fermentors and are now scheduled for process development.

The FCRC fermentaion research program is to explore new or expand certain areas which are not being thoroughly evaluated by contractors and are as follows:

a. Microbial biotransformation of antibiotics, plant material, and synthetic chemicals which have demonstrated limited activity, toxicity, solubility problems, etc. will be evaluated.

b. Evaluation of ecologically different organisms such as thermophiles, halophiles, etc. as potential producers of antineoplastic agents.

c. Isolation of unique microbes which utilized unusual substrates.

d. Co-metabolisms of (a) and (c) will be evaluated.

e. Continuous fermentations of known producers of antineoplastic antibiotics will be evaluated as a method to reduce the costs of the process and as a technique to select better strains of antineoplastic producing organism

f. Fixed culture fermentation of various antineoplastic-producing organisms to produce novel materials will be studied.

g. Stimulants and enzyme inhibitors will be evaluated to see if the metabolisms of organisms is radically changed in hopes of producing different antibiotics.

h. Development of several enzyme products which are of interest to the program.

Future Plans

Three groups of organisms have been shown to be the principal producers of antibiotics; they are bacteria, fungi, and actinomycetes. There are still multitudes of new and different organisms to be isolated using unusual substrates, radical environmental conditions and various unique soils and isolation techniques. In addition, various fermentation and genetic techniques should stimulate the production of more and unique antineoplastic antibiotics. Two of these techniques have been introduced into our contract fermentation program; co-metabolism and biotransformation. Their possibilities are limited only by the ingenuity of the microbiologist. Already more than 1,100 organisms which can utilize unusual substrates have been isolated.

There are several areas of fermentation that have not been explored for their ability to produce new antineoplastic materials. Among these are (a) the use of a variety of different carbon sources for fermentation substrates and as co-oxidizing materials which we are now pursuing; (b) the study of yeasts, of thermophilic and psychrophilic microbes, and of mixed cultures which will be evaluated in the near future; and (c) systematic evaluation of the microbial world of various substrates.

CHEMICAL AND DRUG INFORMATION SECTION

DRUG DEVELOPMENT BRANCH

Scope

This Section, which resulted from the consolidation of the Data Processin and Computer Systems Development Section and the Scientific Records Sec is responsible for : (1) implementing the automated Chemical Informatio System (CIS) which supports the accessioning, storage, and distributio chemicals and drugs acquired for antitumor testing and the retrieval of i formation from the data base; (2) operating the CIS, including the coord nation of staff and contract activities related to the CIS; (3) managing the storage and retrieval within the Drug Development Branch (DDB) of ch ical information in both automated and manual files; (4) developing mode using quantitative structure activity determination methods to establish priorities and to select the optimal test system for antitumor screening; and (5) providing support to literature surveillance effort to identify c pounds of interest which are reported in the literature and in various chemical files maintained by federal agencies, industrial organizations, information venders.

Staff

The Section's staff consists of three chemists, two system analysts, one mathmetician, one chemical information clerk, and four supporting cleric staff.

Emphasis of Effort

During this report period, the Section concentrated on: (1) installing t final phase of the CIS, the training the Drug Research and Development P gram (DR&DP) staff and contractors interacting with the system, and oper the expanded system; (2) reviewing the system documentation effort by th system implementation contractor (University of Pennsylvania) to assure transfer of knowledge to the system operations contractor (Chemical Abst Service); (3) providing chemical information to DR&DP decision-makers; experimenting with statistical and pattern recognition methods in correl chemical fragments with biological activity to develop a model for predi antitumor activity in potential accessions; and (5) determining the feas bility of systematically monitoring the literature to identify compounds reported biological characteristics which would warrant acquisition for tumor testing.

Chemical Information System

The final phase of the system was installed at the Chemical Abstracts Se (CAS) in February, 1976. The first phase of the system handles a wide r of chemical and drug accession activities, including the identification duplicates, assignment of accession numbers, and the output of various r including chemical structures, required by DR&DP staff and contractors. final phase extends the system by providing shipping procedures, invento

control, and the transfer of data to the Biology System regarding shipments and replenishment actions.

In early 1976, tests were conducted to install the structural identification feature of the system. This feature provides for the computerized inspection of all new accessions to identify compounds of interest to the Program. This feature may be extended to identify and to defer testing of compounds in classes which have yielded a low rate of active compounds or compounds in classes which have been adequately studied.

By automating many manual procedures, the CIS has accelerated the accessioning process and has provided improved operational and management control over the contractors engaged in the accessioniong activities. During 1975, the system registered and assigned new NSC numbers to 21,294 acquisitions. At the end of 1975, the system had retrievable chemical structure records and related data for compounds assigned 274,879 unique NSC accession numbers.

Automated Retrieval of Information

The Inquiry subsystem, a component of the CIS developed under contract by the University of Pennsylvania, provides access to the files for full chemical structure search and substructure search, including the retrieval of associated non-structural information. This capability has resulted in improved response time to queries with more comprehansive results, and has served as a valuable tool in investigating quantitative structure activity determinations.

Nomenclature Support

In July, 1975 the routine assignment of systematic nomenclature to all accessions was discontinued. A 28,500-page name and molecular formula index (containing 482,756 names) was produced by computer output microfilm technique to permit compact storage and easier access. Thereafter, on-demand nomenclature support for systematic retrivial names was provided.

In April, 1976 a new NCI Drug Development Name Index will be computer-generated by algorithmically selecting non-systematic chemical names for NCI compounds from the DR&DP Bibliography (and other name) Files and from the Chemical Abstracts Service (CAS) Registry Nomenclature File for all compounds reported in the literature. This index provides approximately 750,000 names frequently referred to as author names, common names, trade names, and trivial names with and NSC accession number. The index will permit a chemical substance to be identified by a variety of names commonly used by investigators in the fields of chemistry, biochemistry, and medicine. When a structure is provided, the DR&DP Chemical file may be interactively queried to determine whether the compound had been acquired for screening. The CAS Registry Number associated with the name will facilitate the retrieval of information from the literature. The machine-preparation of this new index was feasible because the DR&DP chemical file is in the same format as the CAS Registry file.

Preselection Projects

With the need for maximum selectivity in acquiring compounds to be screened

during 1976, increased effort was dedicated to develop improved methods to preselect compounds with a greater than historic probability of activity. Specifically methods are being investigated how best to: (1) select compounds with new structural features previously unstudied; (2) emphasize compounds in promising structural classes and deemphasize adequately studied or non-productive classes; (3) identify compounds worthy of acquisition which: (a) were developed through chemical, biochemical, cell cycle or kinetic, and pharmacological rationales; or (b) have demonstrated biological activity in non-NCI systems and programs; and (4) assign priorities to compounds selected for testing, including the selection of the test system(s) most likely to demonstrate activity.

Progress has been made in the quantitative structure activity determination project using molecular structure fragments used to query the DR&DP CIS file and screening data from the DR&DP Biology file. Most of the programming has been completed and several experiments were run on a small data set taken from the literature. A training set of 136 compounds screened in the ependymoblastoma test system was used to separate actives from inactives among 34 compounds. The results which were statistically significant compare favorably with results reported by other investigators which were derived from two pattern recognition methods, i.e., learning machine and nearest neighbors. Unlike these latter methods, the technique used for this preselection project is geared to large amounts of data. Experimentation is continuing to remove the redundancy in and increase the relevance of the molecular structural fragments used. Chemical and biological data for some 19,000 compounds tested in P388 lymphocytic leukemia and L1210 lymphoid leukemia be used shortly to determine whether this approach is valid in an operational environment. (See Notice of Intramural Research Project Z 01 CM 017101-01 for details.)

With the support of the Chemical Abstract Service, automated methods are being actively explored to identify in the literature compounds which have displayed biochemical or other biological properties which would indicate a high probability of antitumor activity. The structures for compounds of interest in the literature are matched by automated methods with compounds in our collection to identify duplicates. The criteria for preselection based on biological characteristics are being formalized and preliminary searches appear to be yielding good results.

The Section served in a technical supportive role in organizing and participating in two of a series of meetings with outside consultants to establish improved methods in preselecting compounds. A computerized analysis of the DR&DP collection was made by searching approximately 70 substructures of interest to determine: (1) the total number of compounds in each class in the file; (2) the number of actives in each class; and (3) for the actives, the test systems which demonstrated activity. These data were presented at the October, 1975 preselection meeting.

At the May, 1976 preselection meeting: (1) data will be presented on compounds in classes tentatively designated as inactive or adequately-studied; (2) preliminary restults will be reported on investigating a method to identify compounds among potential acquisitions which would be biologically equivalent to compounds previously testd; (3) results of the project in

using computer methods to preselect compounds based on quantitative structure activity determinations will be reviewed; and (4) a presentation will be made on the findings in investigating automated literature surveillance methods to identify compounds of interest to the Program.

In the coming year, the development effort will be concentrated on analyzing the file to develop improved preselection techniques, completing the feasibility study on systematic literature surveillance, assuring that the documentation of the implemented DR&DP chemical information system is completed by the University of Pennsylvania in a manner to facilitate system maintenance by the Chemical Abstracts Service (CAS), and the monitoring of system operations and enhancements by CAS.

TABLE

CONTRACTS MONITORED BY THE SECTION

CONTRACTOR	PRINCIPAL INVESTIGATOR	CONTRACT NO.
Chemical Abstracts Service	Seals	NO1-CM-43722
University of Pennsylvania	Lefkovitz	NO1-CM-33719

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07101-02 DDB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Computer Methods for Drug Preselection Based on Structure-Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Hodes	Research Mathematician	DDB NCI
OTHER:	G. Hazard	Chemist	DDB NCI
	S. Richman	Head, Chem. & Drug. Info. Sec.	DDB NCI
	R. Geran	Biologist	DEB NCI
	J. Waters	Head, Automated Info. Sec.	DEB NCI
	K. Chu	Chemist	CG DCCP
	A. Feldman	Research Chemist	WRAMC
	J. Page	Chemist	WRAMC
	M. Shapiro	Mathematician	DCRT NIH
	R. Jernigan	Biophysicist	LTB DCBD

COOPERATING UNITS (if any)
Walter Reed Army Institute of Research, Department of Army
Chemical Abstract Service
University of Pennsylvania

LAB/BRANCH
Drug Development Branch

SECTION
Chemical and Drug Information Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

SUMMARY OF WORK (200 words or less - underline keywords)

Computerized quantitative structure activity determinations over a broad range of chemical structures were investigated. A new heuristic method was designed to establish priorities and to select the optimal test system for antitumor screening. This method, based on the statistical significance of molecular structure fragment (keys) was applied on a relatively small set of compounds in a variety of chemical classes. The results were statistically significant but considerable additional modification and testing on more and larger data sets is needed. The priority assignment must also satisfy the more stringent test of applicability in an operational environment. Experimentation is continuing to remove redundancy and increase the relevance of the structural fragments. Work on a large-scale model is in progress. Biological data in a machine-processable form are available in the Drug Research and Development screening program for hundreds of thousands of compounds in mouse tumor systems. Molecular structural keys that have been used to query the Drug Research and Development chemical information structure file are similarly available for these compounds. The results of this larger test are expected to be conclusive and, hopefully, useful.

PROJECT DESCRIPTION

Objectives:

1. Evaluate the potential of structure-activity determinations over broad ranges of compounds for specific biological test systems.

2. Demonstrate the feasibility of such methods to preselect compounds for antitumor screening, e.g., in setting priorities for screening or in selecting appropriate test systems.

3. Demonstrate the feasibility that such methods may be used to identify additional promising compounds or compounds inadequately represented in the file.

Methods Employed

1. Strict criteria for activity and inactivity of compounds were established. Active compounds were defined as having passed decision network Stage II. Inactive compounds were defined as having been used in at least two regimens in the given test system, each regimen having at least three dose levels, non-toxic, with at least two injections in the treatment, none of the regimens showing the increased life span required for minimum activity. Also, inactives were collected from only the more recent half of the file. Using these criteria, sets of active and inactive compounds in each of three test systems (3LE21, 3PS31, and 3B131), were obtained by querying the automated DR&D Biology File.

2. The use of molecular structure fragments that were previously developed as keys for querying the automated DR&D Chemical File are being employed as predictor variables.

3. Each of the fragments is given a weight, heuristically dependent on the statistical significance of its incidence in active compounds and in inactive compounds in a given test system. A new compound is evaluated by summing the weights of its fragments. Thus, we get its scores for activity and inactivity.

4. Statistical and probabilistic methods of reducing redundancy and increasing relevance among the fragments are being explored.

5. Priority is assigned from the basic scores for each compound by first finding the Fisher direction, which gives optimum separation of the actives and inactives of the training set. The unknowns are then projected onto a line in this direction, resulting in a linear order or priority score. The Wilcoxon rank test is used to verify significance.

Current Status

As of this writing, the methods have not yet been fully explored. Most of the programming has been done and several experiments run on a smaller data set taken from the literature. A training set of 136 compounds screened in

the ependymoblastoma test system was used to assign priorities to 34 compounds. Our results compare favorably with results reported by other investigators derived from two pattern recognition methods, i.e., learning machine and nearest neighbors. Unlike these latter methods, our technique is geared to large amounts of data. Further results should be available upon the imminent delivery of the chemical structure data for a large scale test of compounds tested in P388 lymphocytic leukemia and L1210 lymphoid leukemia.

Proposed Course

Results over the next year will determine the directions of future work. If our methods allow good separation of actives and inactives on the basis of molecular structure keys, then we will concentrate on the implementation of such a scheme. If our results turn out to be inadequate, other methods will be used, including: (1) other kinds of structure features; (2) complete sets of three dimensional features found by means of autocorrelation of an electron density map of the molecule; and (3) the addition of physio-chemical parameters (measured and calculated) as other variable features. There are also other, more sophisticated chemical features that may apply each to certain classes of compounds. These methods are sure to be more expensive and elaborate but may still be cost-effective.

Publications

Feldman, A. and Hodes, L.: An Efficient Design for Chemical Structure Searching. I. The Screens., Journal of Chemical Information and Computer Sciences. 15: 3,1975, pp. 147-152.

Hodes, L.: Selection of Descriptors According to Discrimination and Redundancy. Application to Chemical Structure Searching., Journal of Chemical Information and Computer Sciences. (In press)

ANNUAL REPORT

DRUG EVALUATION BRANCH

DRUG RESEARCH AND DEVELOPMENT PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Drug Evaluation Branch (DEB) mission is to develop and coordinate laboratory projects for the recognition, recommendation, and preclinical development of improved therapies against human cancer. This is achieved by (a) screening, de novo, large numbers of synthetic and natural products for biological activities indicative of clinical utility; (b) evaluation, in experimental models of pertinence, materials of interest reported from other testing programs and in the published literature; (c) evaluation of "active" drugs for conditions of usage which may optimize their efficacy - e.g., modifications of treatment schedules, combination chemotherapy, and combined modality treatment; and (d) conduct of fundamental and developmental research aimed at devising improved methods for preclinical drug evaluation.

Implementation is through contracts with highly qualified research institutions. During calendar year 1975, DEB directed research projects at 19 institutions (15 in the U.S.A., 4 foreign) under 27 contracts funded at approximately $10,000,000/a (Table 1). Experimental methods emphasized the use of rodent-tumor models in vivo and mammalian cell cultures with a smaller effort in biochemical and anti-viral screening. The scope of DEB activities (Table 2) encompasses all aspects of preclinical drug evaluation except investigations of direct sites of drug action, physiological distribution, and toxicology. Nevertheless, because of its unique capacity to conduct large, comprehensive animal experiments, DEB collaborated actively with the Laboratory of Experimental Chemotherapy (LEC), The Laboratory of Medicinal Chemistry and Biology (LMCB), and with The Experimental Therapeutics Program (ET), DCT to investigate mechanisms of drug action, pharmacological bases for therapeutic efficacy and drug interactions. Collaboration with the Office of the Associate Director, DR&DP, was aimed at developing required material resources (e.g., animals); with the Drug Development Branch on studies related to rational drug design, selection of compounds for screening, development of "active" natural products, and biological quality control of experimental to final clinical formulations; and with the Office of the Director, DCT, The Cancer Therapy Evaluation Program (CTE), and The Baltimore Cancer Research Center (BCRC) to determine the predictive value of animal models for the treatment of specific human cancers.

The scope of the Branch's experimental approaches was broadened in accord with the expanded objectives of DCT, i.e., the development of improved total therapy. Emphasis was placed on surgery plus chemotherapy, but there was substantial progress in the development of models for radiation plus chemotherapy. Pilot studies to judge the potential value of hyperthermia as a potentiator of drug activity were initiated. Implementation of major changes in drug screening practice, described in last year's report was begun, facilitated by the suc-

cessful development of animal models of specific human tumors and fundamental studies demonstrating the applicability of human tumor xenografts to screening. Progress toward full implementation will depend on development of material and laboratory resources.

The end result of DEB activities is the recommendation of potentially improved therapies against human cancer to DCT Decision Network (DN) groups, clinical groups, and presentation of major findings to the scientific community through publications. Data on the efficacy of new drugs is forwarded to the Investigational Drug Branch (ID), CTE for presentation to the Food and Drug Administration and in clinical brochures. DEB goals and experimental approaches are directed to the objectives of DCT, NCI, and the National Cancer Plan, and to elucidating scientific principles that may impact upon these objectives.

DEB includes the Office of the Chief (J.M. Venditti), the Automated Information Section (J.F. Waters, Head) and the Screening Section (B.J. Abbott, Head). As a result of DCT reorganizations during the last year, the Molecular Biology and Methods Development Section was transferred from DEB to LMCB and the Biochemistry Section was abolished. At present, DEB is staffed by 11 senior professionals including one Commissioned Officer and one Senior Staff Fellow; two junior professionals; and ten technical and clerical personnel. Three additional Visiting Fellows are detailed to DR&DP intramural laboratories.

In CY 1975, primary screening was initiated on 39,370 materials - 30,979 synthetic compounds and 8,391 natural product extracts. The synthetic compounds first tested in 1975 included 912 synthesized by DDB contractors as congeners of clinical compounds or because of other specific interest. A total of 33,607 screening tests were performed in tumor bearing rodents; a test is a treated group of 3-10 animals. Screening of synthetic agents accounted for 61% of the in vivo tests with 39% devoted to screening of natural products, although the latter accounted for only 21% of the new material input. Of 110,021 in vivo tests on natural products, 43% involved screening of crude extracts and 57% were conducted to bioassay for activity in conjunction with fractionation leading to isolation of active chemical components. Of 23,643 in vitro tests, 17% involved synthetic materials (mainly compounds submitted in amounts insufficient for in vivo screening plus others whose in vitro activity was of special interest), and 83% involved crude natural products and their fractions. The relatively great proportion of in vitro testing devoted to natural products is a consequence of the practice of screening crude extracts in vivo and in vitro and using the less expensive in vitro test as a bioassay tool where correlative activity exists. Program statistics reported herein were summarized from management reports generated by the Value Engineering Company (33706) which develops and maintains the DEB biological data file, and include all tests submitted to the automated data processing contractor. For some of our more recently developed models, the data are not yet computer processable. These figures should be interpreted with this in mind.

Through the process of progressive testing and review of data generated in this program as well as from elsewhere (see current report of the Screening Section, DEB, Table 7) 18 materials passed DN-2A as candidates for further development to clinical trial; 10 on the basis of findings in DR&DP contract laboratories,

one on the basis of testing conducted in DR&DP intramural laboratories and confirmed in contract laboratories, and seven on the basis of test results from other programs. For two of the latter, DEB test results bore substantially on the decision to move ahead, and we are currently investigating all 18 materials in detailed studies pertinent to Branch functions. The following listing of materials passing DN-2A in CY 1975 includes a brief statement of the specific basis for selection of each. Where the sole basis was animal antitumor screening data, this is simply noted and the sensitive animal tumor(cited. Ancillary findings from DEB contract laboratories are noted along with the institution and contract number. Involvement of a contract managed by another DCT unit is so indicated after the contract number. Studies conducted independent of contractual arrangements or non-contract collaborations are indicated by the absence of a contract number.

Chartreusin (NSC-5159) - activity against P388 leukemia and B16 melanoma in DEB screens. Sterigmatocystin (NSC-204985) - activity against P388 and L1210 leukemias in DEB screens. Polyene antibiotic (NSC-208642) - activity against P388 and B16 in DEB screens. Isolate from Bouvardia Ternifolia (NSC-227262) - activity against P388 and B16 in DEB screens. Methanesulfon-M-aniside derivative (NSC-249992) - improved formulation of NSC-141549 with equivalent activit in DEB screens; probably should not be regarded as a new material. Spirogermanium compound (NSC-192965) - reported clinical activity; inactive against L1210, P388, B16, and Lewis lung carcinoma (LL) in DEB screens.

PALA (NSC-224131) - activity against B16 and LL and lack of activity against L1210 and P388 in DR&DP intramural laboratories (R.K. Johnson, LEC) confirmed in DEB contracts; inhibitor of aspartyl transcarbamylase (G.R. Stark, Stanford University School of Medicine).

Thiosemicarbazone derivative (NSC-246112) - activity against L1210 in DEB screens; more potent than parent compound in inhibiting ribonucleotide reductase and less potential for metabolic inactivation in man (A. Sartorelli, Yale University School of Medicine, 53824).

PCNU (NSC-95466) - activity against mouse brain tumors in DEB screens and collaborative screening (with V. Levin, University of California, 67055, BCRC); advantageous Lipid/water partition coefficient for CNS penetration (with C. Hansch, Pomona College, 33703, LMCB).

6-Selenoguanosine (NSC-137679) - activity against L1210 in DEB screens; activity against Mecca and 6C3HED lymphosarcoma which are refractory to 6-Thioguanosine (6-TGR) - confirmed by DEB screens - as well as demonstrated failure of phosphorylation beyond the monophosphate and consequential failure to incorporate into DNA, in contrast to 6-TGR (with A. LePage, supported in part by terminated DEB contract, 22042, Scripps Clinic).

Soluble (+) isomer of ICRF-159 (NSC-169780) - activity in DEB screens equivalent to the insoluble racemate.

Spirohydantoin mustard (NSC-172112) - a bis-chloroethylamine derivative of a known CNS active (not antitumor) structure, designed and synthesized by J. Driscoll, LMCB, as carrier for the alkylating functional group; activity in

DEB screening against intracranially (ic) implanted mouse ependymoblastoma (EM) markedly superior to Cyclophosphamide (CPA), Melphalan (L-PAM), or Nitrogen mustard (Hazelton Laboratories, 57007).

Adriamycin-trifluoroacetyl-valerate, AD-32 (NSC-240131) - activity superior to Adriamycin (ADR) against L1210 (E. Modest and N. Israel, Sidney Farber Cancer Center) - confirmed in DEB screen at Southern Research Institute (SRI, 43756).

Ledacrine (NSC-247561) - reported activity against human ovarian carcinoma and capacity to cure rats with Walker 256 carcinoma (Poland). W256 results confirmed in DEB screen at Arthur D. Little, Inc., (ADL, 57006).

Two nitro-imidazoles, RO-5-0963 (NSC-261036), RO-7-0582 (NSC-261037), and Metronidazole (NSC-69587) - ability to preferentially sensitize hypoxic tumor tissues to radiation; currently being evaluated comparatively as radiation sensitizers at ADL (53765) and The Radiobiological Institute, Netherlands (53763), LEC.

Hematoporphyrin (NSC-59265) - selective uptake into tumor tissue and regression of photo-radiated tumors of treated animals (T. J. Dougherty, Roswell Park Memorial Institute); current collaboration (ADL, 53765) with the Roswell Park group is aimed at providing information needed for the design of protocols for clinical evaluation of NSC-59265 plus photo-radiation.

The brief statements summarizing the bases for acceptance of these agents illustrate the extent of DEB participation in the selection process regardless of the stimulus for interest. It is the DEB mission to utilize its contract laboratory resources to provide data required for the selection of a new drug for clinical trial regardless of the biological, biochemical, or pharmacological basis of initial interest whether it was found de novo in our program or in other programs. In addition, we are committed to the conduct of in-depth quantitative studies to provide information relative to the optimal conditions for drug effectiveness. About 1/3 of the DEB contract funding for in vivo studies is devoted to screening in its most strict sense; about 7% is devoted to natural product bioassays, and the rest to detailed evaluation of active drugs, drug combinations, combined treatment modalities, and the development of improved models for drug evaluation (Table 2).

Of the 18 drugs that passed DN-2A during the last year, eight are structurally related to drugs that have been in clinical trial or in development to clinical trial, or were synthesized on the basis of the known pharmacological activities related to their structural class. Considering the day-to-day logistical problems and the resources required to operate a large-scale screening program; and the expectedly low yield of active materials if the input were entirely "random," it would be ideal if all eventually clinically useful materials could be rationally designed. Historically, this has not been the case. To maintain a reasonable flow of new materials to the clinic and to provide data to those engaged in the synthesis or extraction of active materials, it will be necessary to continue screening at a reasonable level. On the other hand, to accommodate our expanded tumor spectrum (Chart 1), the number of materials entered into the program will be restricted and this input reduction has begun; the 39,370 materials first screened in 1975 represent a substantial carry-over

of 1974 submissions. In order to effect a reduced input in an intelligent way we expect to establish priorities for screening. A high priority will be given to agents which have demonstrated pertinent biological activities in other programs or which are synthesized on a sound rational basis.

During the coming year, the plan to test new synthetic compounds and natural product isolates against a spectrum of models of specific human tumors will be implemented progressively. Although the selection of all systems has not yet been accomplished, it is expected that this panel of animal screens (Chart 1) will include mouse colon, breast, and lung tumors; and human tumor xenografts in athymic mice as material resources to conduct such testing are developed. Other animal tumors of specific interest (e.g., transplantable brain tumors) will be used in specific instances. The effort required to test a drug against the panel of tumors is substantially greater than that required to screen against one rapidly growing transplantable leukemia followed by the testing of active materials against two well standardized transplantable solid tumors according to previous practice. Previous screening procedures essentially required activity against either L1210 (for synthetic materials) or P388 (for natural product isolates) to warrant further testing against slower growing tumors. Although exceptions to this practice were made on an ad hoc basis, no alternative criteria for testing beyond initial screening had been formally established. The plan illustrated in Chart 1 is intended to circumvent this disadvantage, at least in part. The number of models comprising the screening panel, and the increased effort required for some, means that the input to the panel will be limited; the figure of 500-1,000 per year representing an educated guess based on projected capacity. After considering a number of alternatives (see Venditti, J.M., Cancer Chemother. Rep., Part 2, 5: 1-4, 1975), we have determined that the most reasonable approach would be to utilize, as an initial in vivo screen or "pre-screen," one with demonstrated sensitivity to most classes of clinically effective anticancer drugs, but which, nevertheless, is sufficiently discriminating to avoid "overloading" the spectrum. The previous screen for synthetic agents, L1210, while known to be highly predictive for clinical utility, was considered to be too restrictive for a "pre-screen" designed to minimize the chance of missing a compound likely to show activity in one of the tumors in the spectrum. A comprehensive review of existing screening data indicated that mouse leukemia P388 would represent a more appropriate "pre-screen" because (a) its response to drugs of various classes is qualitatively similar to that of L1210, and (b) it is quantitatively more sensitive than L1210. All in vivo screening laboratories have been instructed to conduct initial screening of synthetic materials against P388. The object of the "pre-screen," which must be limited to one in vivo system because of cost considerations, is to select a population of materials which will yield a higher percentage of "actives" in one or more of the spectrum tumors than would de novo screening in the entire spectrum. In addition, it is expected that the "pre-screen" will include an in vitro assay which will permit the selection, for subsequent in vivo spectrum screening, of highly cytotoxic materials and materials which may induce cellular differentiation, a potentially useful drug action which cannot be discerned using the present in vivo models or the KB cell culture screen. A multiwell plate system has been developed for the rapid sensitive determination of both cytotoxicity and induction of differentiation of Friend Erythroleukemia cells by experimental drugs (Yale, 53824), and we are investigating the utility of this model for

large-scale in vitro screening (ADL, 67078).

Crude natural products will continue to be tested against P388 in mice, and in cell cultures - mainly against KB, but also against L1210 and/or P388 cells. Experience in using these cells as bioassay tools indicates no reason to alter this practice. Active natural product isolates will be evaluated in the tumor spectrum.

Despite the necessity of limiting our initial in vivo screen to one assay, we recognize that no animal model is known to predict perfectly for clinical utility. Therefore, we plan to augment the input to the tumor spectrum with materials with antitumor activity reported in the world's literature and from other screening programs, and with compounds reported to exhibit pertinent biochemical and other biological activities. Such a compound will be tested in the DCT "pre-screens" to provide data for future analyses, but will be tested in the tumor spectrum regardless of "pre-screening" results. Because of their demonstrated past utility, L1210 and B16 will be retained as part of the screening spectrum. Lewis lung carcinoma (LL) is still being used, but alternative mouse lung tumor systems are being investigated. Congeners of known active drugs will continue to be tested in the specialized assays designed for each structural class. As materials emerging as active from any of the spectrum models (or even very active in the P388 screen) are subjected to disease-oriented clinical trial, this approach to screening will provide, prospectively, critical information relative to the value of specific animal tumors or combinations of animal tumors. The state of development of various experimental models for broad spectrum screening is discussed in the current report of the Screening Section, DEB; the published Proceedings of the May 1975 Screeners Conference (Cancer Chemother. Rep., Part 2, 5: December 1975); and the forthcoming publication of the Proceedings of the April 1976 Screeners Conference.

As the DCT panel of antitumor screens is phased into use, some pre-selection or priority rating, even for the "pre-screen", may have to be made. Logical bases for pre-selection are under discussion, but it is reasonably certain that, in the absence of demonstrated biological or biochemical data of some kind, structural considerations will weigh heavily. Again, the figure of 15,000 synthetic materials to be entered into the "pre-screen" (Chart 1) is a guess based on plans to provide for screening against the panel (human tumor xenograft screening excepted) within our present screening capacity. Although some developmental work with xenografts has been done under DEB contracts (Chester Beatty Research Institute, CBRI, 43736; Battelle Memorial Institute, BMI, 57005) in DR&DP intramural laboratories, and in collaboration with non-contract supported investigators (Stehlin Foundation for Cancer Research), we must develop contract laboratory capacity in order to utilize these expensive models for moderate-scale screening.

Criteria for pre-selecting, from the literature or on the basis of findings from other programs, materials that may "by-pass" the "pre-screen" as well as for establishing priorities for "pre-screening" have not yet been established. However, some tentative bases, subject to approval of the Director, DCT and the Associate Director, DR&DP have emerged from discussions among the senior staffs of DEB (J.M. Venditti, and M.K. Wolpert), LEC (A. Goldin and R.K. Johnson), LMCB (D.G. Johns, V.H. Bono, and J.S. Driscoll), and DDB (H. Wood

and R. Engle). Summaries of these discussions have been prepared by S. Richman (DDB) and submitted to the Associate Director, DR&DP. Clearly demonstrated antitumor activity in man or in animal models used in other screening programs should be sufficient cause to consider a compound as a candidate to "by-pass" the "pre-screen." In this context, we should consider any valid in vivo antitumor test system as a valid "pre-screen." Through DEB contracts with CBRI, London (43736), The Institute Jules Bordet, Brussels (57040), and the "Mario Negri" Institute for Pharmacological Research, Milan (53720); LEC contracts with Jules Bordet (53840) and the Japanese Foundation for Cancer Research (22054); our active collaborations with other drug evaluation programs within the U.S.A.; collaborations with the European Organization for Research in the Treatment of Cancer; and our active participation in the World Health Organization sponsored project for standardization of screening methodology, the USA-Japanese Joint Working Group, and the USA-USSR Joint Working Group, the number of "by-pass" candidate drugs should not be inconsiderable. However, the structures of these drugs should be reviewed to determine their suitability for broad panel screening, or alternatively, specialized "analog" testing. A number of other reasons for "by-passing" the "pre-screen" have been suggested, but a listing at this time would be premature. Similarly, more than a dozen broad biological, biochemical, and pharmacological activities (other than antitumor activity) were rated with respect to whether they should impart a high or low priority for "pre-screening."

Experimental findings that should channel a material into specialized test models have been identified. For example, drugs that may alter the metabolism of another drug - for combination chemotherapy; immunostimulants - for testing in LEC contracts aimed at this objective; radiosensitizers or protectors - for combined modality studies; drugs which exhibit specific organ toxicities. Drugs known to penetrate the CNS; and antiviral activity. In fact, DR&DP has within its current contract program resources for most of this type of specialized testing although the level of effort is very small for some.

Last year 158 materials were examined for effects on the growth of mouse 3T3 cells in culture, on the transformation of 3T3 cells by murine sarcoma virus (MSV), and/or on reverse transcriptase; and DNA, RNA, and poly (A) polymerizing enzymes (Litton Bionetics, 33741). There was no clear correlation between inhibition of viral reverse transcriptase and inhibition of MSV induced focus formation. Compounds which demonstrate notable antiviral activity in culture are being tested for prophylaxis against viral tumor induction in vivo (SRI, 43756). The project for in vitro antiviral testing is in the process of recompetition with significant changes in its scope. The screen for inhibition of transformation by RNA viruses will be continued since RNA viruses have been associated with leukemia in fowl, cats, and mice. Inhibition of transformation by DNA viruses will be added since DNA viruses have been implicated in certain human malignancies; e.g., Burkitt's lymphoma and cervical cancer. Inhibition of viral reverse transcriptase will be de-emphasized.

Combined modality studies (ADL, 53765) have been done using cobalt-gamma irradiation with six drugs: Actinomycin D (Act.D), L-PAM, and ADR against Ridgway osteogenic sarcoma (ROS), CPA, and 5-Fluorouracil (5-FU) against B16, and BCNU against LL. The three solid tumor animal models were chosen because of particular biological behavior and response to chemotherapy. With ROS, re-

latively large local tumors regressed following local radiation at 900 r/day for five successive days, and small, but established tumors were very sensitive to systemic ADR or Act.D therapy. In the ROS model, Act.D, L-PAM, or ADR combined with radiation (400-600 r/day X 5) was more effective than chemotherapy alone or radiation alone at comparable doses The pattern of tumor regression and regrowth following combined treatment of ROS showed that the model can be used to evaluate multiple courses of therapy that simulate clinical treatment schedules. The LL solid tumor metastasizes; large tumors are relatively resistant to radiation and to most anticancer drugs. Well established LL did not regress following localized radiation although tumor progression was inhibited transiently. A single BCNU treatment did not affect tumor progression or survival time. Radiation (500 r/day X 5) plus BCNU (32 mg/kg X 1) produced a greater degree of LL growth inhibition than did radiation alone and a slight increase (25%) in the lifespan of the mice. Although the overall effectiveness of combined therapy of LL was not impressive, the results indicate that the model should be studied further. It was difficult to assess the advantage of combined treatment using the B16 model because even well established B16 tumors regressed completely following radiation alone treatment. The ADL group is currently evaluating the EMT-6 fibrosarcoma as a model for radiation and combined modality therapy. Studies of the comparative radiosensitization potential of a number of drugs including Metronidazole, RO-7-0582, and RO-5-9963 are underway. The latter three agents are not known to have antitumor activity. However, there are a number of reported radiation sensitizers which are active antitumor drugs per se. Combined therapy with radiation plus active antitumor drugs should be a fruitful area of study because localized radiation plus systemic chemotherapy should not result in additive toxicity to normal cells. However, evaluation of therapeutic efficacy should be based on cure rate of animals with established tumors combined with quantitative in vivo studies of the surviving tumor cell fraction rather than regression or inhibition of local primary tumors. The SRI group (43756) has demonstrated, for several mouse tumors, that a significantly smaller number of viable tumor cells can establish lethal tumors in the presence of radiation-inactivated tumor cells than in their absence. This suggests the possibility that small numbers of residual viable tumor cells in radiation-treated tumor sites may be a greater threat to cure than an equally small number of cells remaining after surgery.

Small tumor cell foci left in situ during primary surgical excision or that escape radiation damage, and distant metastases as well, appear to be the primary cause of failure of surgical or radiation treatment, proper targets for chemotherapy and/or immunotherapy. Current knowledge of tumor cell population growth kinetics indicates that the growth fraction of localized tumors is inversely related to cell population size. Therefore, relatively small micrometases might be expected to be more sensitive to chemotherapy than the cells in the larger primary tumor. Studies (SRI, 43756) with four metastic murine solid tumors - LL, B16, C3H mammary carcinoma, and colon tumor 26 - demonstrated significantly improved cure rates with drug treatment following surgical removal of well established primary tumors. Instances where drug treatment following surgery was substantially more effective than either modality alone are too numerous to list in this report. They are described in the Annual Report of the Southern Research Institute, January 2, 1976 (43756).

The group at Catholic Medical Center (23703) have established the spontaneous CD8F1 mammary carcinoma as a model for evaluation of combined treatment modalities and combination chemotherapy, and first generation transplants are used for screening. Surgical excision of well established spontaneous tumor is followed by treatment with single drugs, drug combinations, or placebo. The slow growth rate of the tumors and long survival time of untreated mice permits the use of repeated courses of drug treatment. The principal parameter of efficacy is the time to regrowth of the primary tumor following surgery. The superiority of the combination of L-PAM, ADR, and 5-FU over any two drug combination or single drug was demonstrated. The combination of CPA, MTX, and 5-FU is currently being studied. It should be noted that while such studies are appropriate for evaluation of the model, they are retrospective to clinical trials. The ultimate value of models for combined modalities will be their ability to select, prospectively, the potentially most efficacious treatment from among alternatives being considered for clinical use. If we are to provide useful information to clinicians who plan combined modality protocols, it is important that our clinical colleagues inform us of the alternative therapies under consideration and of the specific questions to which the clinical studies are directed. The same considerations should be applied to drug combinations.

There are a number of animal tumor models with demonstrated usefulness for determining, not only therapeutic synergism, but the influence of treatment schedule, treatment sequence, and dose ratio as well. They include L1210, P388, and the solid tumor models used for combined modality studies. ROS appears to be an excellent model for the study of drug combinations essentially for the same reasons that make it one of the preferred models for combined modality studies; advanced ROS tumor will regress or be cured by a number of clinically useful anticancer drugs, and it can be staged to various degrees of advancement to permit simulation of response of human tumors to treatment. During the past year therapeutic synergism against advanced ROS was observed for CPA plus Act.D, L-PAM, or 6-MP. Therapeutic synergism of Adenine-arabinoside by adenosine deaminase inhibitors against L1210 and P388 was an interesting new drug development. Potentiation with combinations of alkylating agents was of special interest since L1210 sublines developed resistant to L-PAM, CPA, or BCNU remained sensitive to the alternative two drugs. Potentiation of activity against transplantable mammary carcinomas of mice was observed for ADR plus 5-FU or Vinblastine (VLB). ADR plus VCR was modestly potentiating.

Conduct of the "analog" screening program during the past year is described in the current report of the Screening Section. Since initiation of this project at ADL (53765) in October 1974, 1,315 agents, synthesized for the most part by DDB contractors have been tested. The objectives of the project are to test congeners of high interest drugs in experimental protocols based on the activities of the parent compound and to provide direct feedback of results to the synthesis contractor to aid in directing further synthesis. We have attempted to control the level of effort in order that it not compromise combined modality tasks conducted under the same contract. Significantly, 47% of the compounds tested were designated as "regulars," i.e., they were synthesized by contractors, but were not readily identified as structural congeners of parent drugs. The yield of active materials from the "regulars" is somewhat higher than the yield from "random" synthetics, but does not approach the very high yields among true congeners of known active drugs which, themselves, pre-

sent an important problem. It is our position that a drug congener ought to demonstrate antitumor activity in an appropriate screen to warrant further development. In the past, quantitatively superior activities in animal tumor models which are sensitive to the parent drug have generated clinical interest in the congener. However, a difference in spectrum of activity would be of greater importance. Studies of activities against tumor variants developed for resistance to the parent drug provide information on whether the congener is a true biochemical analog of the parent, or rather, a uniquely acting drug. The scope of DEB activities does not permit the comparative toxicological and pharmacological evaluation of active analogs that might aid in selection. ET, DCT has established a limited number of contract and intramural projects directed to specific drug classes. For example, a number of water soluble congeners of cis-Pt-II are being evaluated with respect to their relative nephrotoxicity (with J. Ward, ET). A number of active anthracyclines are being tested in models designed to compare their cardiomyopathic potential (with D. Young, ET).

In other structure-activity studies, more than 50 carbohydrate and lipid analogs with the potential to affect membrane-mediated functions were evaluated for cytotoxicity in the Friend Erythroleukemia (Yale, 53824). In collaboration with Dr. K. Folkers (University of Texas), Coenzyme Q analogs, with demonstrated activity in his in vitro test for inhibition of mitochondrial electron flow, were tested in vivo in the IP-W256 model. Of 32 Co-Q analogs tested, 13 produced long-term survivors, a valid indicator of activity in this rather sensitive model. NSC-252188 and 220334 were the most effective.

In continuing studies of correlations between animal tumor activity and clinical usefulness, animal screening data was refined and reanalyzed for 45 compounds which have been in clinical use against human solid tumors (ADL, 33727). At the same time, in collaboration with ID, CTE, the results of clinical trials of the same compounds were re-assessed. The object of the study was to apply multivariate statistical methods to the screening and clinical data and derive formulas which describe the animal model or combination of models which predicts best for individual human tumor types. Data for five animal models (IP-L1210, SC-L1210, P388, B16, and LL) and 17 human solid tumor types were included. The analysis of clinical results probably represents the most comprehensive summary of single drug efficacy in existence. The multivariate discriminant models for each human tumor type describe the best correlation between animal and human responses possible with the data available, although the clinical data was deficient in quantity for some human tumors and some drugs. The results showed that using animal data from the five assays in concert, the probability of predicting a false negative drug and false positive drug, respectively, for human cancer are 4.3% and 21.8% for breast carcinoma, 18.1% and 11.9% for colon cancer, 23.9% and 28.1% for lung cancer, 15.9% and 30.5% for melanoma, and 11.2% and 25.8% for human GI cancer. It is not surprising that the correlations were best for diseases which clearly respond to a number of drugs (e.g., breast carcinoma) and less good for human tumors which respond less well to chemotherapy. Nevertheless, the results of this first mathematically based correlation study were gratifying and a high priority will be given to its continuation. Immediate tasks will be to conduct a similar study for human leukemias and lymphomas, and to continually update the studies as new clinical experiences are reported. In addition, it will be important

to test the clinically evaluable drugs against the new animal screens that are being initiated. For each human tumor the best predictive formula is likely to change as data on new clinical trials and new animal models are generated. Nevertheless, the use of the formula, at any point in time, will result in a number of immediate advantages to DCT. It will define (a) the human tumor(s) most likely to respond to new active drugs, (b) human tumors which are likely responders to older drugs which have not been adequately evaluated against them, (c) animal tumor models which contribute little to the prediction formula for a specific human tumor, and (d) animal models which closely simulate each other. We envision that the further development and use of such analytical procedures will result not only in improved clinical therapy, but also in a more efficient use of clinical and laboratory resources. While no single experimental model, in vivo or otherwise, predicts perfectly and absolutely for activity against human cancer, we recognize that no alternative to the use of non-human experimental systems exists. Therefore, it is incumbent on us to select models that provide the best tools for realization of our objective. Screening is a dynamic undertaking, the models used in this program have changed considerably over the years, and we expect that they will continue to improve progressively.

Table 1

Contracts Monitored by Drug Evaluation Branch - January 1976

Contract NO1-CM-	Institute
33727	Arthur D. Little, Inc. (OC)*
53765	Arthur D. Little, Inc. (OC)
57006	Arthur D. Little, Inc. (SS)
67078	Arthur D. Little, Inc. (SS)
57005	Battelle Memorial Institute (SS)
57108	Baylor College of Medicine (OC)
23703	Catholic Medical Center (SS)
43736	Chester Beatty Research Institute (OC)
57007	Hazelton Laboratories (SS)
43755	Illinois Institute of Technology Res. Inst. (SS)
57004	Illinois Institute of Technology Res. Inst. (SS)
63832	Illinois Institute of Technology Res. Inst. (SS)
(PL-480)	Indian Cancer Research Centre (SS)
57040	Institute Jules Bordet (SS)
33741	Litton Bionetics (OC)
33720	Mario Negri Institute for Pharmacological Res. (SS)
57003	Mason Research Institute (SS)
67077	Miami University of (SS)
43756	Southern Research Institute (OC)
67075	Southwest Foundation for Research and Education (SS)
33706	Value Engineering Company (AIS)
53821	Value Engineering Company (AIS)
53770	WARF Institute, Inc. (SS)
67076	Wisconsin, University of (SS)
33711	Yale University School of Medicine (OC)
53824	Yale University School of Medicine (OC)
57029	Yale University School of Medicine (OC)

*OC = Office of the Chief, SS = Screening Section, AIS = Automated Information Section.

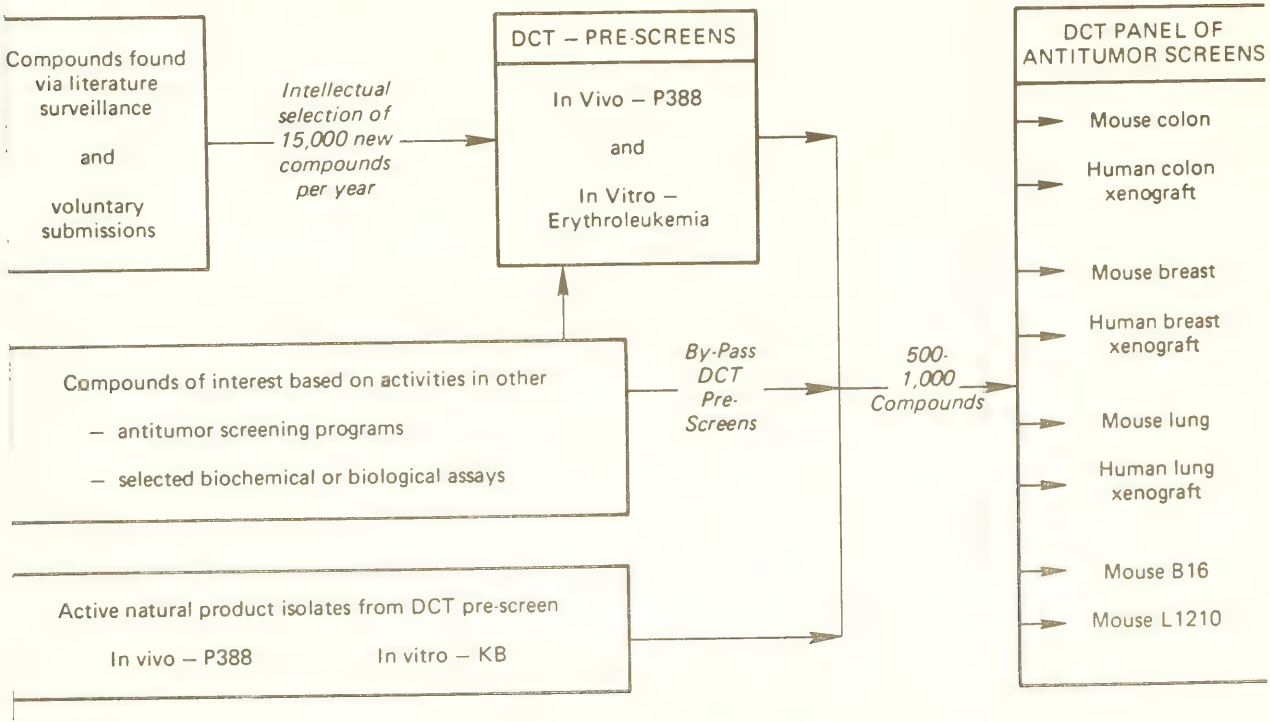
Table 2

DEB Contract Funding by Activity - January, 1976

	Activity	($1,000)
I.	Animal-Tumor Assays	7,675
	Primary and Secondary Screening	2,910
	Natural Product Bioassay	703
	Analog Screening	450
	Detailed Drug Evaluation	1,177
	Combination Chemotherapy	876
	Combined Modalities of Treatment	685
	Human Tumor Xenografts	24
	Methods Development	850
II.	In Vitro Assays	1,128
	Mammalian Cell Culture Screening	402
	Natural Product Bioassay	207
	Microbiological (excluding antiviral)	30
	Antiviral Screening	273
	Biochemical Screening	100
	Cell Kinetics	20
	Methods Development	96
III.	Others	1,364
	Clinical Pharmacology	7
	Compound Collection	31
	Rational Design and Synthesis	33
	Support Activities	1,293
	TOTAL	10,167

Chart 1

Proposed Flow of Compounds Through DCT Screens



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AUTOMATED INFORMATION SECTION

DRUG EVALUATION BRANCH

DRUG RESERACH AND DEVELOPMENT PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Automated Information Section (AIS) is responsible for the implementation and maintenance of the biological data processing system for the Drug Evaluation Branch (DEB), thereby providing for the automated processing, storage, and retrieval of biological data generated in testing laboratories under contract to the DEB, Drug Research & Development Program (DR&DP). Synthetic and natural products are tested in animals for antitumor activity and in cell cultures at various screening laboratories subsequent to entry into the syste By means of the biological activity or inactivity reported, decisions are mad concerning the direction of compound acquisition, synthesis, fractionation of active crude natural products, and the recommendation of new agents for development toward clinical trial. The biological data base is capable of bein queried by special programs thus providing special summary data for: 1) eval uation of the existing screens; 2) design and evaluation of new test systems 3) review of compounds being considered for Division of Cancer Treatment (DCT Decision Point 2A; 4) further evaluation of individual compounds and drug combinations; 5) statistical information required for effective program management.

The AIS is staffed by two professionals, one statistical assistant, one programmer, one computer aide, and one secretary. Professional staff serves as Project Officers and has responsibility for two contracts: Value Engineering Co. (NO1-CM-33706) which provides the major service for the biological data processing system, and Value Engineering Co. (NO1-CM-53821) which maintains the DCT clinical drug distribution and inventory system. During the past year, an increased portion of staff time was devoted to providing special reports, (queries), to the DCT staff members, contractors, and suppliers. The number of such queries increased from 142 in 1974 to 175 for calandar year 1975. The AIS staff has analyzed, designed and implemented a new schedual dependency system. During the year 1975, 29 schedule dependency summarie and plots were done. Following the implementation of this system the AIS staff will initiate an analysis of the Natural Products Processing System. All of the various inputs and outputs of this system are sorely in need of revamping. This will be a very significant undertaking due to the limited size of the staff involved.

The current contract (NO1-CM-33706) for the operation and maintenance of the biological data processing system has been in effect since July 1972. Production costs (both contractor and the Division of Computer Research and Technology) were increased slightly from $2.01 per test line in 1974 to $2.03 in 1975.

The single drug portion of the Front End Transaction Input Processing System was completed, tested, and began production in November, 1975. Revisions of other Front End Systems, including Combination Chemotherapy, Management Information, and Terminal Handler Systems will be completed by the close of the report period.

Microfilming of the Natural Products File as well as the Synthetics File and Control Card Listings was completed in 1975. This was a significant task since the Natural Products File has not been microfilmed since the current contract has been in effect and new programs had to be developed and documented.

A decrease in the number of primary screening tests and an increase in new tumor system testing (much of which is not yet computer processed) resulted from a change in screening philosophy. This shift in emphasis resulted in 14% fewer computer processed tests (table 1). At the same time, however, there was an increase in the amount of systems analysis and programming. Some of the new screening assays are sufficiently complex to require the use of off-line files. These messages are for the Ridgeway Osteogenic Sarcoma, Colon Tumors, B16 Solid Tumor, Antiviral, and the AKR Spontaneous Tumor Tests. New programs were developed for P388 and L1210 In Vitro data. The Southern Research Institute (NO1-CM-43756) solid tumor data processing system was converted to operate in conjunction with the DEB biological data processing system. Work has been initiated on the adaption of the Southern Research Institute system to handle data from the CD8F1 mammary carcinoma screen as used at the Catholic Medical Center (NO1-CM-23703).

Development of a Terminal Handler System which will provide a vehicle for preparing and transmitting screening data for entry into the In Vivo Control Pack Processing System has begun. The scope of the system encompasses functions presently involved in the transcribing of test data results from laboratory notebooks onto NIH forms, submission of the forms to the data processor, preparation of these data for ultimate entry into the Transaction Input Processing System, and its subsequent return as edited data for error correction. An additional improvement of data discrepancy handling involved preparation of a new form for recording testing data in the laboratories. The use of this form will simplify data entry by reducing duplication of entries. Rows and columns will be clearly delineated on the new form, thereby reducing the likelihood of errors in recording information.

In conjunction with the above improvements will be an analysis and design plan for the reformatting of the biological data processing system data base. This will necessitate the rewriting of various update programs and print programs. Also planned for the year 1976 is a redesign of the Screening Data Summary. These planned changes and additional changes necessitated by the dynamic nature of the DEB Screening Program will be done within the anticipated budget.

The Clinical Drug Distribution & Inventory System operated under contract (NO1-CM-53821) was expanded. Daily, monthly and quarterly reports were prepared on schedule and 104 special reports were produced. In order to objectively verify all drug orders against protocol requirements, an automated

drug order verification system was developed. Increased automation will be added to this system as the need arises.

TABLE 1

DIVISION OF CANCER TREATMENT PROGRAM STATISTICS

	Calendar Year 1975	Calendar Year 1974	Calandar Year 1973	Calandar Year 1972
1. Materials Added to the Master Files*				
Synthetic Compounds	26,511	29,946	26,906	14,781
Selected Agents	175	107	214	-
Plants	6,393	5,375	11,066	10,434
Fermentation Products	1,328	718	2,550	2,712
Animal Products	2,086	1,675	3,034	1,669
Total	36,493	37,821	43,770	29,596
2. In Vivo Antitumor Tests Completed and Processed**				
L1210 Lymphoid Leukemia	160,089+	211,832	134,562	103,681
P388 Lymphocytic Leukemia	125,958+	124,396	198,723	180,027
Walker Carcinosarcoma	1,318	909	1,704	2,770
B16 Melanoma	8,837	11,724	16,989	6,028
Lewis Lung Carcinoma	5,847	2,770	2,441	4,141
AK Leukemia	462	925	-	-
Other Tumors	3,723	2,821	2,696	6,458
Total (In Vivo Antitumor Tests)	306,234	355,377	356,115	303,105
3. In Vivo Toxicity Tests	5,554	9,215	6,430	2,900
4. Cell Culture	32,931	35,371	31,820	25,996
Alkaloid	350	500	450	-
Biochemical Assay	28	-	337	231
Total Non In Vivo	33,309	35,871	32,607	26,227
Total of ALL Tests	345,097	400,463	395,152	332,232

* These figures are derived from the Drug Research and Developement Biological Data Processing System and differ from those generated by the Drug Development Branch, which logs in materials when NSC numbers are assigned. The 36,493 materials added to the Master Files in 1975 is less than the 39,370 materials actually tested for the first time in 1975 (see report of the Screening Section, DEB, table 4). This is a consequence of the lag between the day that a screening laboratory acknowledges receipt of a material and the day that the experiment is

actually begun. Since all screening laboratories purposefully maintain a moderate backlog of materials, some materials actually tested in 1975 had been added to the Master File in 1974.

** The number of *in vivo* antitumor tests and *in vitro* tests shown in this table for 1975 represents the total of completed tests reported and processed regardless of when the test was initiated. The number of tests shown in the report of the Screening Section, (tables 1-3) are those that were begun in 1975.

+ Includes tests against Resistant Tumor Lines-971 in L1210 variant tumors and 306 in P388 variant tumors.

SCREENING SECTION

DRUG EVALUATION BRANCH

DRUG RESEARCH AND DEVELOPMENT PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Screening Section has as its principal objectives the selection and recommendation of new drugs as candidates for development toward clinical trial against cancer and the investigation of means for increasing the therapeutic usefulness of known antitumor drugs. Objectives are attained by (a) screening a large number of synthetic and natural products in vivo, and to a limited degree, in vitro; (b) conduct of biological assays for directing fractionation of natural products and isolation of active components; (c) in-dept evaluation of materials which emerge from primary screens with emphasis on the influence of factors such as route and treatment schedule that affect therapeutic efficacy; (d) analysis, evaluation, and presentation of pertinent experimental results to Division of Cancer Treatment (DCT) Staff; and (e) methodology research leading to development of improved assays for drug evaluation. Program is implemented through establishment and coordination of work conducted by contractual arrangement with qualified institutions, and through collaborations with DCT intramural laboratories. Activity against experimental tumors in vivo is the principal basis for recommending candidate drugs for development toward clinical trial and for studying optimal conditions for drug usage. In vitro activity against mammalian tumor cells or microorganisms provides a means of recognizing biological activity among synthetic agents submitted in insufficient quantity for in vivo screening, for following fractionation and isolation of active components of crude natural products with correlative in vivo activity, for providing information relative to mechanisms of drug actions, and for developing assays for estimating effective drug levels in blood and tissues.

I. Staff Functions

The Screening Section is currently staffed by six senior professionals, one on detail from the office of the Chief, DEB, one junior professional, and five technical and clerical personnel. Principal investigators are: B.J. Abbott (Head); M.C. Baratta (CO); R.I. Geran; N.H. Greenberg; M.M. Macdonald, and N.R. Melnick.

Staff serve as Project Officers for eight contracts devoted predominantly to presumptive in vivo screening; Arthur D. Little, Inc. (NO1-CM-57006), Battelle Columbus Laboratories (NO1-CM-57005), Hazleton Laboratories (NO1-CM-57007), IIT Research Institute (NO1-CM-57004), Institute Jules Bordet (NO1-CM-57040), "Mario Negri" Institute for Pharmacological Research (NO1-CM-33720), Mason Research Institute (NO1-CM-57003), and WARF Institute (NO1-CM-53770). Although the number of contractors con-

ducting predominantly in vivo screening increased by one, the average test capacity per contract as well as our total in vivo test capacity has been reduced because of program changes and cost inflation. The in vitro screening contracts were competed. Eleven proposals were received and five awards were made: Arthur D. Little, Inc. (NO1-CM-67078), IIT Research Institute (NO1-CM-63832), Southwest Foundation for Research & Education (NO1-CM-67075), University of Miami (NO1-CM-67077), and University of Wisconsin (NO1-CM-67076). The Screening Section has Project Officer responsibility for a contract with Catholic Medical Center (NO1-CM-23703) for evaluation and use of the CD8F1 mouse mammary tumor as a model for human breast carcinoma and for production of CD8F1 mice, and collaborates with the Chief, Drug Evaluation Branch (DEB); Chief, Drug Development Branch (DDB); Chief for Laboratory Research, Drug Research and Development Program (DR&DP); and the Associate Director, DR&DP to monitor screening, detailed drug evaluation, and methodology tasks performed under contracts with Southern Research Institute (NO1-CM-43756), Arthur D. Little, Inc. (NO1-CM-33727 and 53765), Upjohn Company (NO1-CM-43753), Michigan Dept. of Health (NO1-CM-33702), Institute Jules Bordet (NO1-CM-53840), Japanese Foundation for Cancer Research (NO1-CM-22054), The Chester Beatty Research Institute (NO1-CM-43736), and Research Triangle Institute (NO1-CM-92019). A Screening Services Contract with IIT Research Institute (NO1-CM-43755) assists staff in the management of screening data which has become increasingly complex as protocols have been modified and expanded in recent years.

By reviewing biological effects of natural product fractions, the Screening Section establishes directions of effort under additional DR&DP contracts for natural product fractionation and isolation. Staff monitor the work of contract laboratories to insure that tasks are carried out as scheduled and with a high degree of scientific and technical proficiency and that projects are directed to the overall goals of DR&DP, DCT, NCI, and the National Cancer Plan. The staff select and design assays for uncovering of new active drugs, design experimental protocols for their use, review results, recommend candidate drugs for clinical trial, and prepare data for publication and for review by the Drug Evaluation Committee, Operating Committee, Experimental Therapeutics Area, Clinical Trials Area, and for DCT Decision Network review.

II. Significant Accomplishments

A. Determination of Antitumor Activity of New Agents

Primary screening in vivo consists of the initial testing of synthetic and natural products submitted to DR&DP for activity against transplantable mouse tumors. Screening protocols are designed to uncover a modest number of materials which are then subjected to progressively exacting requirements designed to select those with the greatest clinical potential.

During this fiscal year, the Division of Cancer Treatment placed increased emphasis on the testing of fewer materials against a larger spectrum of animal models of specific human tumors. A program has been initiated to utilize leukemia P388 in mice as a prescreen to aid in the selection of agents for broader spectrum screening. P388 was selected because its response to most clinically effective drugs is qualitatively similar to that of L1210 and also because it is quantitatively more sensitive than L1210. The prescreen will also include an *in vitro* assay to select for subsequent broad spectrum *in vivo* testing, highly cytotoxic materials and materials which may induce cellular differentiation. This type of drug action cannot be discerned using the *in vivo* or *in vitro* test systems presently utilized. Currently, the Friend virus induced erythroleukemia *in vitro* is being studied for this purpose.

In addition to materials which show activity in our pre-screens, materials which have demonstrated interesting activity outside our program (e.g., antitumor activity in other screens, and other pertinent biochemical or biological activities) or because of structural considerations, will also be tested against a panel of secondary *in vivo* screens. The precise composition of the secondary screening panel has not yet been determined and it may undergo changes as new information becomes available. However, it will include mouse colon, breast, and lung tumors and human tumor xenografts of these types grown in athymic mice. Other tumors such as brain, renal, ovarian or bladder may be used on a selective basis or as special interests warrant. Tumor systems will be phased into the panel as permitted by the completion of developmental work, the availability of laboratory capacity, and in the case of human tumor xenografts, the availability of athymic (nude) mice. The panel will include leukemia L1210, B16 melanoma and Lewis Lung carcinoma. Alternative lung tumor models are being studied. The effort required to adequately test a compound in the panel of secondary screens will be much greater than that required for follow-up testing of active materials in the past. Therefore, the number of materials to be tested in this panel will be limited (probably not more than 500-1000 per year), and this, in turn, will require a considerable reduction in the input of new materials to the pre-screen.

Materials regarded as potential candidates for clinical trial are studied for route and schedule dependency. Analogs of compounds already in development are compared with the parent compound in test systems selected on the basis of the biological activity of the paren Crude natural products continue to be tested both *in vitro* (KB) and against the P388 in mice. Where activity is observed in both, the less expensive cell culture assay is used for bioassay to follow activity during the process of fractionation.

Tables 1-3 show the number of screening tests conducted and computer processed during calendar year 1975. An *in vivo* test is defined as

a treated group of from three to ten animals, and an in vitro test consists of a concentration - response assay from which the cytotoxic ED50 is extrapolated. Of 307,250 total tests (Table 1), 283,607 were in vivo, and 23,643 were in vitro (Table 3). Tables 1-3 also show the number of tests conducted with compounds selected for further study because of activity in initial screening (SAC), other synthetic compounds (Regular), structural congeners of known active compounds (Analogs), crude plant extracts, crude fermentation beers, crude animal extracts, and natural product fractions. The numbers in Tables 1-3 do not include testing in new test systems under current development or other testing which is not yet computer processable.

Table 4 shows, by type, the number of materials first tested in any test system during 1975. It also gives an analysis of the average lag-time between the date a test is initiated and the date the test results are computer processed. The total, 39,370, is the number of materials for which testing was initiated in 1975 and processed by February 19, 1976. The average lag-time is greatest for selected agents and markedly lower for other synthetics or natural products. In general, primary screening is completed in a relatively short time. As materials are determined to be active, or require specialized testing they are moved to the selected agents (SAC) file. The longer lag-time for the latter is a reflection of both activity and more sophisticated test systems requiring more time for completion.

B. Selection of Candidate Drugs for Clinical Trial

During calendar year 1975, 529 new compounds passed initial screens (Table 5). Of these, 326 (62%) demonstrated confirmed activity in vivo against the tumors listed. The remaining 203 compounds, active against KB cells in culture, were either inactive in vivo or not tested in vivo because of limited supply. The Data Review Subcommittee (DRS) reviewed screening results for 1,100 compounds, and referred 126 compounds to the Drug Evaluation Committee (DEC). Eighteen compounds passed the DCT-Linear Array Decision Network point 2A (DN-2A) and proceeded in development toward clinical trial (Table 6,7).

C. Detailed Evaluation of New and Old Drugs of Particular Interest

The Screening Section is responsible for the detailed evaluation in rodent tumor systems of drugs which passed DN-2A including route and schedule dependency, biological quality control of experimental clinical formulations, toxicity in non-tumor bearing rodents, and other appropriate studies to determine the extent to which modifications in drug usage might increase therapeutic efficacy. Results are reviewed and summarized for Decision Points subsequent to DN-2A, for inclusion in clinical brochures and IND applications, and for presentation to clinical working groups. Combination chemotherapy and combined modalities of treatment are evaluated in collaboration with the Office of the Chief, DEB.

The Selected Agents (SAC) file comprising comprehensive records of experimental results for all active drugs and compounds of special interest, maintained by the section, contains information on 6,225 materials. Approximately one-fourth of these are being actively pursued on their own merit and the others are included in comparison studies with related drugs. New data received from this program, other DR&DP contract or intramural laboratories, non-NCI sponsored drug evaluation programs, and the literature is reviewed and when warranted, these materials are presented to the DN-2A group.

1. Route and Schedule Dependency Studies Twenty-seven schedule dependency studies were initiated during 1975: 14 in L1210, 10 in P388, and three in other assays. Follow-up schedule dependency testing was conducted as required. The Screening Committee reviewed data from 29 schedule dependency studies and submitted summary reports to the Chief, DEB and for use in clinical brochures. Schedule dependency studies usually involve three routes of administration and seven treatment schedules. The principal drug route is intraperitoneal; for highly water-insoluble compounds, however, the oral route is emphasized.

2. Combination Chemotherapy An offline computer file of animal data for combinations of drugs is maintained and includes a total of over 1,300 drug combinations that have been tested in rodent models; some in several models. We have followed three approaches in planning animal experimentation in this area: 1) combinations known to be clinically useful to assess the degree to which enhancement of clinical efficacy is paralleled in animal models; 2) drug combinations of current clinical interest to determine the optimal conditions for their use with regard to dosage ratio, treatment schedule, and sequence of administration; and 3) untried combinations of clinically-active drugs to identify promising combinations prospectively. Experimental studies are both disease and drug oriented. DEB is currently analyzing all contract generated combination chemotherapy studies in collaboration with the Laboratory of Experimental Chemotherapy.

3. Biological Quality Control of Experimental Clinical Formulations

In collaborating with the Clinical Drug Distribution Section, DDB, comparative testing of bulk drug and various experimental clinical formulations was conducted for seven agents in development to clinical trial. Such testing, which may require the evaluation of a number of experimental forms for each drug, is necessary to insure against loss or reduction of antitumor efficacy during the process of formulation.

4. Screening of Structural Congeners of Active Drugs This project is part of the DCT approach to "analog" development and entails (a) identification by DCT of drug classes of special interest,

in particular the major classes of clinically active drugs; (b) acquisition, mainly thru DDB contracts, of new structural congeners and other rationally designed drugs; and (c) biological, biochemical, toxicological, and pharmacological comparative testing of congeners to determine potential superiority over the parent drug or uniqueness of action. In vivo testing of "analogs" was conducted at Arthur D. Little, Inc. (NO1-CM-53765) using animal-tumor models selected on the basis of the qualitative and quantitative activity of the parent compound. While the main objective of this project is to develop congeners with potential clinical superiority, it also provides information to aid in the selection of approaches to new synthesis. Materials are tested within one week of receipt in the laboratory and results are sent directly to the supplier of the material. Approximately 20% of over 1200 compounds tested in the "analog" screening program showed activity, and 56% of the active materials represented seven compound classes - derivatives of Camptothecin (NSC 100880), Actinomycin D (NSC 3053), Anthracyclines, melphalan (NSC 8806), cis-Pt-II (NSC 119875), DTIC (NSC 45388), and Cyclophosphamide (NSC 26271).

D. Methodology (Research and Development)

Initiation of the plan to test agents of interest against a panel of experimental tumors as potential models for specific human malignancies was described earlier. In all contracts sponsored by DEB, a part of the effort was devoted to research aimed at the development of new and improved assay systems. A substantial number of materials has been examined in the transplantable mouse tumor models, but only a small number has been tested against transplantable human tumor xenografts. The development of each system as a useful screen for hundreds of materials can be looked upon as a four-phased project. (1) The scientific validity of the approach must be determined in fundamental studies. These are often conducted outside the contract mechanism (e.g., intramurally or by grants) but may be supported by DEB contracts when the information required is not available through an alternative mechanism. (2) Developmental research based on fundamental findings is aimed at determining the operational characteristics of the model (e.g., kinetics of tumor growth, transplantability, constancy of morphological and growth characteristics over many transplant generations, responses to known drugs, reproducibility, etc.). The end-products of developmental research are a determination of the usefulness of the model (e.g., for large-scale screening, specialized screening, combination chemotherapy, combined modality therapy) and the description of precise experimental protocols for its use including the parameters of and quantitative criteria for therapeutic activity. (3) For models determined to be appropriate as a large-scale screen, laboratory capacity to conduct testing at the desired level must be developed. The Screening Section expects to be able to carry out screening in new transplantable animal tumor models within its

existing contract laboratory capacity. However, the capacity to screen against human tumor xenografts does not exist and should be developed within the next year. (4) Material resources must be developed (e.g., a sufficient supply of athymic mice for xenograft screening). Although certain specific models have been identified as appropriate for our panel of secondary screens, the selection of all systems has not yet been accomplished. The panel may be expanded or modified as a consequence of future laboratory and clinical results and as new models are developed. Specialized models (e.g., animal brain tumors) will be used for selected drugs.

The selected studies described below were conducted in the following laboratories. Unless otherwise noted the contracts were monitored by Screening Section Staff and the contract numbers are prefixed by NO1-CM.

Arthur D. Little, Inc. (ADL) - 53765, Office of the Chief, (O.C.), DEB and 57006.
Battelle Memorial Institute (BMI) - 57005.
Baylor College of Medicine (BCM) - 57108, O.C., DEB.
Catholic Medical Center (CMC) - 23703.
Chester Beatty Research Institute (CBRI) - 43736, O.C., DEB.
Hazleton Laboratories - 57007.
IIT Research Institute - 57004.
Mason Research Institute (MRI) - 57030, Laboratory of Experimental Chemotherapy (LEC), DR&DP and NO1-CB-31146, Breast Cancer Task Force, NCI.
Stehlin Research Foundation (SRF) - no contract - collaboration with DEB and LEC.
Southern Research Institute (SRI) - 43756, O.C., DEB.
Tumor Biology Section, LEC, DR&DP - intramural.

1. Human tumor xenografts

CBRI used T-lympnocyte deprived (by thymectomy and radiation) mice to carry transplantable xenografts from human tumors and to determine their sensitivity to clinically active drugs. Hexamethylmelamine (HMM) was active against xenografts of human bronchial carcinoma, lung oat cell carcinoma, and kidney carcinoma. Other drugs showing activity against xenografts of human carcinomas were cyclophosphamide (CPA) - ovarian, Melphalan-ovarian, BCNU - colon, 5-Fluorouracil (5FU) - colon. A human astrocytoma implanted subcutaneously into conditioned mice responded to radiatio therapy and to CCNU. While subcutaneous xenografts of human brain tumors can provide information on the sensitivity of the tumor cell type to drugs, therapeutic efficacy in the patient will depend on the drug's ability to traverse the "blood-brain barrier" as well. MRI, SRF (collaboration with B. Giovanella), and the Tumor Biology Section, LEC (with A. Goldin and R. Johnson) established human tumor xenografts in serial transplantation in athymic

("Nude") mice in order to study the transplantability and biological characteristics of the models. BMI has been studying xenograft models, developed mainly at SRF, from the view point of their potential usefulness as predictive systems for screening. We hope to establish a contract resource for such screening within the next year. In addition, we should develop a bank of frozen human tumors and provide a resource for periodic assessment of tumor viability and constancy of biological and biochemical characteristics. MRI maintained a bank of frozen animal and human mammary tumors for the Breast Cancer Task Force, NCI. Both the MRI and SRF groups demonstrated that human tumor cells can be propagated and maintained in vitro, and transferred to animals without loss of lethal potential.

2. Transplantable animal tumors

A number of contract laboratories and the Tumor Biology Section, LEC, showed the feasibility of intravenous implantation of Lewis Lung carcinoma to assess drug effectiveness against small tumor foci in the lung. The mouse squamous cell carcinoma, developed by P. Nettesheim, Oak Ridge National Laboratory, is being evaluated as a potential secondary screen at ADL. The tumor was shown to be quite resistant to therapy, being insensitive to radiation, Methotrexate (MTX), CPA, Vinblastine (VLB), Adriamycin (ADR), Actinomycin D (Act. D), Procarbazine, Colchicine, DTIC, and Daunomycin (DAUN). Although some degree of inhibition of local tumor growth was observed, none of the therapies increased the survival of the mice.

CMC and SRI continued to use the spontaneous mammary carcinoma of CD8F1 mice. The system was developed at CMC, presently the sole source for breeding the animals. First generation transplants are used for screening. SRI continued to use the C3H mammary carcinoma. Both the CD8F1 and C3H tumors are sensitive to a high percentage of drugs of known clinical usefulness against human breast tumors. Transplanted C3H tumors and spontaneous CD8F1 tumors are being used to evaluate combined modality therapy: i.e., surgery and chemotherapy. The CD8F1 model appears to be suitable for the study of multiple modality therapy including immunotherapy. BCM is developing a murine mammary tumor system characterized by a transplantable preneoplastic cell population, the hyperplastic alveolar nodule, which gives rise to mammary tumors. The system is being studied as a potential model for determining drug activity against neoplastic transformation as well as primary tumors.

SRI developed, as transplantable tumor lines, a number of carcinogen induced mouse colon tumors. Two are being considered as secondary screens. Colon tumor number 38, a well differentiated polyploid carcinoma of C57B1/6 mice, metastasizes in about 40% of the mice. Number 26, an undifferentiated colon tumor of BALB/C

mice, metastasizes in about 70% of the animals. Some differences among the carcinogen induced tumors with regard to drug sensitivity were observed, but so far, they have tended to respond to the same drugs - 5FU, its deoxyribonucleoside (5-FUdR), nitrosoureas, and CPA appear to be the most active drugs tested. Mitomycin C, Cytosine arabinoside (Ara-C), Dibromodulcitol (DBD), Dianhydrogalactitol (DAG), ADR, and VLB were less active. HMM, Vincristine (VCR), and MTX were inactive. These colon tumor models appear to be quite useful for surgery plus chemotherapy studies. For example, against advanced tumor 26, surgery plus MeCCNU and 5FU cured 50-60% of the animals as contrasted to a cure rate of less than 10% for surgery alone and none for combination chemotherapy alone. The question of the unique usefulness of animal models of specific human tumors as screens or assays for combined modality studies cannot be answered until we accumulate a great deal more data in the new tumor systems especially with unknown drugs. Almost all of the drugs tested against these colon tumors, and other "new" models as well, have demonstrated activity against animal tumors that have been used regularly over the last five years. It is noteworthy, for example, that surgery plus MeCCNU cured 70% of mice with established SC implanted B16 melanoma while surgery alone cured only 20% of the mice, and drug alone cured none.

Ridgway osteogenic sarcoma (ROS) has been shown at SRI to be sensitive to many clinically active drugs. Established tumors regressed under treatment with Act. D, ADR, DAUN, Bleomycin, CPA, Melphalan, 5-FU, Ara-C, 6-Mercaptopurine (6-MP), VCR, and Cis-Pt-II. ROS, therefore, promises to be a useful experimental model for chemotherapy trials, especially with drug combinations, on which selected clinical treatment schedules and treatment sequences might be based. Therapeutic synergism was observed with CPA plus Act. D, ADR, 6-MP, or 5-FU; and ADR plus Cis-Pt-II. With the combination of CPA plus Melphalan, therapeutic potentiation was observed when Melphalan was given prior to CPA but not when the treatment sequence was reversed. ADL showed ROS to be sensitive to radiation and that the combination of radiation plus Act. D, Melphalan, or ADR was more effective than radiation alone or drug alone at equivalent dosages.

IIT further evaluated the mouse renal cell carcinoma described by Murphy and Hrushesky (J. Natl. Cancer Inst. 50: 1013, 1973). ADR, VLB, MeCCNU, and BCNU demonstrated activity against this tumor. However, the long survival time of untreated mice may preclude its use except on a very selected basis.

Hazleton Laboratories has used an intracranially transplantable mouse ependymoblastoma, originally described by Zimmerman and Arnold (Cancer Res. 1: 919, 1941) and developed further by Ausman et al (Cancer Res. 30: 2394, 1970) as an aid in the development of improved therapy against human brain tumors. The

tumors were exquisitely sensitive to nitrosoureas, DAG, and the spirohydantoin mustard (NSC 172112). The latter compound was synthesized by J. Driscoll, Laboratory of Medicinal Chemistry and Biology, DR&DP in an attempt to add anticancer active functional groups to a CNS penetrating drug. The activity of NSC 172112 in this model is substantially greater than the moderate activity of other alkylating agents. DAG, the active *in vivo* metabolite of DBD was curative whereas DBD produced only a moderate increase in survival time.

Over the last year, increased emphasis was placed on the development of models which may be useful for combined treatment modality studies and secondary screens as well. Some recent studies combining drug treatment with surgery or radiation were described in the foregoing discussion. The effort in these studies is expected to increase now that DCT is involved in the development of drugs as radiation sensitizers and photo-sensitizers regardless of their inherent antitumor activity. L. Van Putten (Radiobiological Institute TNO, Contract NO1-CM-53673 with LEC, DR&DP) and the ADL group under contract NO1-CM-53765 represent DR&DP's present resource for radiation studies.

Clearly, some of the models described above are in the early stages of development while others have been used to a moderate extent. Some will be used regularly as secondary screens while others will be restricted to very special uses. Space limitations preclude a detailed account of these as well as other, more preliminary, models. More detailed descriptions will be found in the published "Proceedings" of the May 1975 "Screeners' Conference" (Cancer Chemotherapy Rep., Part 2, Vol. 5, Dec., 1975) and the forthcoming publication (Cancer Treatment Reports) of the April, 1976 conference.

III. Related Projects

The need for uniformity among contract laboratories in a large screening program requires continued surveillance. It is the responsibility of the Screening Section to assure uniform biological characteristics of tumor lines as well as host animals. To achieve this, frozen tumor banks are maintained by several contract laboratories from which new lines are dispensed periodically to screening laboratories.

The need for host animals in good health and in large numbers necessitates close liaison with the Mammalian Genetics and Animal Production Section, DR&DP, whose contract laboratories perform studies related to the diagnosis and control of animal disease and monitor the quality of the animals utilized in the program.

The Screening Section, as the major user of the DR&DP data processing

system, continues to work closely with the Automated Information Section, DEB by advising and assisting in the planning of modifications and the monitoring of data necessary to an ongoing data processing system.

IV. Proposed Course of Projects

The Screening Section will continue to supervise the testing and evaluation of data for all materials screened in the DR&DP program. This includes the continuous evaluation of the primary screen and its logistics, criteria, and tumor models. Research and methodology will be carried out under contract for related projects of interest to DCT, NCI. Research projects are aimed primarily at devising new or improved antitumor assays, evaluation of new test systems for their potential as screening tools, and the development of standardized methodology to be used by the participating contract laboratories.

TABLE 1

NUMBER OF IN VIVO AND IN VITRO SCREENING TESTS CONDUCTED IN CALENDAR YEAR 1975*

	Selected Agents (SAC)		Other Synthetics		Natural Products			
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total
1st Quarter	9,190	1,309	38,179	2,265	9,072	3,441	1,836	14,349 (Crude)
					11,918	1,602	2,796	16,316 (Fraction)
					20,990	5,043	4,632	30,665 (Both)
2nd Quarter	8,751	1,994	31,947	2,468	7,681	3,426	1,694	12,801 (Crude)
					13,527	2,980	3,438	19,945 (Fraction)
					21,208	6,406	5,132	32,746 (Both)
3rd Quarter	6,838	1,518	36,598	2,249	10,830	2,472	1,240	14,542 (Crude)
					12,506	4,293	4,063	20,862 (Fraction)
					23,336	6,765	5,303	35,404 (Both)
4th Quarter	5,563	1,171	25,684	1,819	12,384	3,332	1,520	17,236 (Crude)
					9,324	1,730	2,602	13,656 (Fraction)
					21,708	5,062	4,122	30,892 (Both)
Total	30,342	5,992	132,408	8,801	87,242	23,276	19,189	129,707 (Both)
			Total Crude Only -		39,967	12,571	6,290	58,928
			Total Fractions Only -		47,275	10,605	12,899	70,779

Total for all Tests	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter	1975
	81,608	77,906	82,607	65,129	307,250

* Includes test begun in 1975 and reported to the Automated Data Processing (ADP) contractor by February 19, 1976.

TABLE 2

NUMBER OF IN VIVO SCREENING TESTS CONDUCTED IN CALENDAR YEAR 1975*

	Selected Agents (SAC)		Other Synthetics		Natural Products			
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total
1st Quarter	8,906	1,309	36,761	2,265	6,273	3,197	1,826	11,296 (Crude)
					10,689	1,377	2,787	14,853 (Fraction)
					16,962	4,574	4,613	26,149 (Both)
2nd Quarter	8,366	1,994	31,071	2,468	6,239	2,985	1,574	10,798 (Crude)
					11,825	2,161	3,430	17,416 (Fraction)
					18,064	5,146	5,004	28,214 (Both)
3rd Quarter	6,741	1,518	36,320	2,249	7,778	1,956	767	10,501 (Crude)
					11,140	3,344	4,059	18,543 (Fraction)
					18,918	5,300	4,826	29,044 (Both)
4th Quarter	5,354	1,171	25,274	1,819	10,609	2,831	1,207	14,647 (Crude)
					8,221	1,147	2,599	11,967 (Fraction)
					18,830	3,978	3,806	26,614 (Both)
Total	29,367	5,992	129,426	8,801	72,774	18,998	18,249	110,021 (Both)
			Total Crude Only -		30,899	10,969	5,374	47,242
			Total Fractions Only -		41,875	8,029	12,875	62,779

	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter	1975
Total for all In Vivo Tests	75,390	72,113	75,872	60,232	283,607

* Includes test begun in 1975 and reported to ADP contractor by February 19, 1976.

TABLE 3

NUMBER OF IN VITRO SCREENING TESTS CONDUCTED IN CALENDAR YEAR 1975*

	Selected Agents (SAC)		Other Synthetics		Natural Products			
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total
1st Quarter	284		1,418		2,799	244	10	3,053 (Crude)
					1,229	225	9	1,463 (Fraction)
					4,028	469	19	4,516 (Both)
2nd Quarter	385		876		1,442	441	120	2,003 (Crude)
					1,702	819	8	2,529 (Fraction)
					3,144	1,260	128	4,532 (Both)
3rd Quarter	97		278		3,052	516	473	4,041 (Crude)
					1,366	949	4	2,319 (Fraction)
					4,418	1,465	477	6,360 (Both)
4th Quarter	209		410		1,775	501	313	2,589 (Crude)
					1,103	583	3	1,689 (Fraction)
					2,878	1,084	316	4,278 (Both)
Total	975		2,982		14,468	4,278	940	19,686 (Both)
			Total Crude Only -		9,068	1,702	916	11,686
			Total Frantions Only -		5,400	2,576	24	8,000

	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter	1975
Total for all In Vitro Tests	6,218	5,793	6,735	4,897	23,643

* Includes test begun in 1975 and reported to the ADP contractor by February 19, 1976.

TABLE 4

SUMMARY OF MATERIALS FIRST TESTED IN ANY SYSTEM IN 1975*

Quar.	Selected Agents (SAC) Synthetic	Analogs	Total	Other Synthetics Synthetic	Analogs	Total	Natural Products Plants	Animal	Ferment.	Total	Total
1	106	31	137	8443	159	8602	1869	131	343	2343	11,082
2	52	22	74	7256	204	7460	792	439	283	1514	9,048
3	56	31	87	8673	246	8919	1085	479	435	1999	11,005
4	17	2	19	5464	217	5681	1508	594	433	2535	8,235
	231	86	317	29,836	826	30,662	5254	1643	1494	8391	39,370

Analysis of lag time between the date test initiated at the laboratory (date-on) and the date the test results are processed, reveals that the following percentages of the data have been processed in the second, third, and fourth months after the date-on month:

Month Beyond Date-On	Selected Agents (SAC) Average	Range	Synthetics Average	Range	Natural Products Average	Range
Second	24%	12%-42%	56%	27%-80%	63%	38%-84%
Third	67%	57%-75%	93%	89%-96%	97%	95%-99%
Fourth	91%	79%-99%	97%	94%-99%	99%	96%-100%

* Includes compounds for which test results were processed by February 19, 1976.

TABLE 5

NEW ADDITIONS TO THE SELECTED AGENTS LISTING

CALENDAR YEAR 1975

Total Number of Materials	Total Number for Each Tumor System	
326	202	P388 Leukemia
	110	L1210 Leukemia
	6	Ependymoblastoma (IC)
	4	Walker 256
	1	L1210-IC Leukemia
	2	B16 melanoma
	1	Lewis Lung
203	203	KB cell culture

TABLE 6

STATUS OF ACTIVE COMPOUNDS

CALENDAR YEAR 1975

New Actives	529
No. of compounds reviewed by	
DRS DEC	1,100
DEC	126
Total reviewed	1,226
Passed DN2	18
Route & schedule dependency experiments scheduled	27
No. of schedule dependency studies reviewed by Screening Committee	29

TABLE 7

COMPOUNDS ASSIGNED TO DN-2 FOR THE FIRST TIME IN 1975*

NSC No.	NAME	DATE OF ASSIGNMENT
5159	Chartreusin, dihydrate	February 24
59265	Hematoporphytrin	December 1
69587	Imidazole-1-ethanol, 2-methyl-5-nitro-	December 1
95466	Urea, 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitroso-	December 1
	PCNU	
137679	9H-Purine-6(1H)-one, 2-amino-9-β-D-ribofuranosyl-6-seleno-	October 6
	Guanosine, 6-seleno-	
169780	2,6-Piperazinedione, 4,4'-(1-methyl-1,2-ethanediyl)bis-, (+)-	February 24
172112	1,3-Diazaspiro[4.5]decane-2,4-dione, 3-[2-[bis(2-chloroethyl)amino]ethyl]-	July 21
	Spirohydantoin Mustard	
192965	2-Aza-8-germaspiro[4.5]decane-2-propanamine, 8,8-diethyl-N,N-dimethyl-, dihydrochloride	February 24
204985	Sterigmatocystin	July 21
208642	Polyene antibiotic similar to Filipin a Pentaene	July 21
224131	L-Aspartic acid, N-(phosphonoacetyl)-, tetrasodium salr	July 21
	PALA	
227262	Compound from Bouvardia Ternifolia	January 13

TABLE 7 Cont'd.

NSC No.	NAME	DATE OF ASSIGNMENT
246112	Hydrazinecarbothioamide, 2-[(5-amino-4-methyl-1-isoquinolinyl)methylene]-	October 6
246131	Adriamycin, N-Trifluoroacetyl, 14-valerate	October 6
247561	1,3-Propanediamine, N,N-dimethyl-N'-(1-nitro-9-acridinyl)-, dihydrochloride	December 1
	Ledacrine	
249992	Methanesulfon-m-anisidide, 4'-(9-acridinylamino)-, mono-hydrochloride	January 13
261036	1,2-Propanediol,3-(2-nitro-1H-imidazol-1-yl	October 6
261037	1H-Imidazole-1-ethanol, α-(methoxymethyl)-2-nitro-	October 6

* The bases on which the agents were entered into development to clinical trial are discussed in the current report of the Chief, Drug Evaluation Branch.

ANNUAL REPORT

LABORATORY OF EXPERIMENTAL CHEMOTHERAPY

DRUG RESEARCH AND DEVELOPMENT PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

The laboratory conducts fundamental research in experimental chemotherapy with broad programs in tumor biology, biochemical pharmacology and immunochemotherapy. The programs in tumor biology includes such areas as investigation of the molecular basis of drug specificity, structure-activity relationships in important classes of antineoplastic agents, the fundamental basis and therapy of invasiveness and metastasis, models for multiple combination chemotherapy based on biologic and biochemical parameters of drug action, resistance and cross-resistance to antineoplastic agents, and development of solid tumor systems for drug evaluation. The program in biochemical pharmacology includes studies of the effects of important new agents at the cellular level, relationship of protein synthesis inhibition to cytotoxicity, and mechanism of resistance and action of established and potential antineoplastic agents. The immunochemotherapy program includes investigation and development of screening and evaluation systems for immune suppression and immune enhancement, investigation of therapy-related immunologic phenomena and selected immunochemotherapy studies in drug chimerism, adoptive immunity plus chemotherapy, humoral antibody plus chemotherapy, antigenic alteration plus chemotherapy, immunogenetics and chemotherapy and immunochemotherapy of viral-induced tumors.

The Laboratory of Experimental Chemotherapy currently includes the Office of the Chief (A. Goldin) and two sections: Tumor Biology (R. K. Johnson) and Immunochemotherapy (D. Houchens).

Two Individual Project Reports are included this year for the Laboratory of Experimental Chemotherapy:

(a) Tumor Biology and Chemotherapy, Project No. ZO1-CM-07100-03

(b) Immunochemotherapy Studies in Various Tumor Systems, Project ZO1-CM-03577-03

During this year of operation of the Laboratory of Experimental Chemotherapy the two activated sections, Tumor Biology and Immunochemotherapy, have made a number of significant advances including: demonstration of antitumor activity of PALA, a rationally synthesized inhibitor of de novo pyrimidine nucleoside biosynthesis; the establishment of a program of drug evaluation in athymic nude mice bearing human colon carcinoma xenografts; and demonstration of antitumor activity of Corynebacterium parvum, alone and in combination with cytotoxic drugs, in transplantable syngeneic tumor systems widely used for drug evaluation. In addition, several new contracts were initiated during the year for evaluation of immunosuppression and immunotherapy, development of resistant cells to drugs of interest to the Program, and development of new solid tumor systems, particularly human tumor xenografts, for drug evaluation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-07100-03 LEC

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Tumor Biology and Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Randall K. Johnson	Actg. Head, Tumor Biology Sect	LEC	NCI
OTHERS:	Abraham Goldin	Actg. Chief, Lab. Exptl. Chemo.	LEC	NCI
	David P. Houchens	Senior Staff Fellow	LEC	NCI
	Artemio A. Ovejera	Visiting Fellow	LEC	NCI
	Toshio Inouye	Visiting Fellow	LEC	NCI
	Makoto Inaba	Visiting Fellow	LEC	NCI
	Manik P. Chitnis	Visiting Fellow	DEB	NCI
	Rainhardt Osieka	Guest Worker	LEC	NCI

COOPERATING UNITS (if any)

NONE

LAB/BRANCH

Laboratory of Experimental Chemotherapy

SECTION

Tumor Biology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.0	5.0	3.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to develop new chemotherapeutic approaches to cancer by studying new agents and biological systems for evaluation of potential antineoplastic agents. The topics of current interest are: 1) antitumor activity of actinomycin and anthracycline antibiotics; 2) biochemical pharmacology of potential antineoplastic agents including PALA, maytansine and inhibitors of protein biosynthesis; 3) resistance to anthracycline antibiotics and Vinca alkaloids; and 4) chemotherapy of human colon carcinoma xenografts.

PH-6040
(12-75)

Objectives:

To undertake fundamental studies in therapy of experimental tumors; to investigate potential and established clinical cancer chemotherapeutic agents with respect to their effect on malignant growth and selectivity; to determine the relationship between various biological properties of tumors and response to chemotherapy; to study structure-activity relationships among major classes of antineoplastic agents; to develop underlying principles of successful cancer therapy. Specific objectives include: study of drug sensitivity differences and control mechanisms in the cell cycle; study of the origin and basis of invasiveness, metastasis and tumor angiogenesis and the effects of drugs on these phenomena; investigation of means for effective therapy and prevention of emergence of resistant cell populations; study of the mechanism of drug action and mechanism of drug-induced cell death; study of the influence of drugs on hematopoietic and other sensitive host tissues in relation to antitumor effect; development of new and improved biological systems for screening and detailed evaluation of potential antineoplastic agents; determination of underlying principles of effective combination chemotherapy and combined modality treatment.

Methods Employed: Standard laboratory procedures were employed.

Major Findings:

I. Structure-Activity Relationships and Analogue Evaluation

A. Actinomycins

A continuing effort to find an actinomycin with a broader spectrum of activity yielded several analogues which appear to be as active as actinomycin D in a number of experimental tumor systems but none, as yet, that show distinct superiority over the parent compound. Two compounds which showed confirmed but marginal superiority over actinomycin D in one tumor system, actinomycin $Pip_{1\beta}$ (superior in ip B16 melanoma) and azetomycin I (superior in iv Lewis lung carcinoma), are being evaluated extensively in a spectrum of tumors.

B. Anthracyclines

A major effort was initiated in comparative evaluation of activity of anthracycline antibiotics in experimental tumor systems. The semisynthetic derivative developed at Sidney Farber Cancer Center, N-(trifluoroacetyl)-adriamycin-14-valerate was evaluated in comparison to adriamycin against L1210 leukemia, P388 leukemia and a subline of P388 leukemia resistant to adriamycin. We could not confirm the published reports of superior antitumor activity of this analogue and found cross-resistance in the adriamycin-resistant tumor. The complex of adriamycin with ferric ion, which does not inhibit Na^+- K^+ dependent ATPase and may, thus, be less cardiotoxic than adriamycin, was shown to be as active as the parent compound against early and advanced P388 leukemia. A number of naturally

occurring and semisynthetic anthracyclines are currently under evaluation in a spectrum of experimental tumors.

C. Antineoplastic Agents in Clinical Use in the Peoples' Republic of China

Two analogues which are in widespread clinical use in the Peoples' Republic of China, nitrocaphane (an analogue of melphalan) and tisupurine (an analogue of 6-mercaptopurine) were obtained for evaluation in experimental tumors. Nitrocaphane proved to be no better than melphalan in L1210 and P388 leukemias or Lewis lung carcinoma. Evaluation of tisupurine is currently underway.

II. Detailed Evaluation of New Potential Antitumor Agents

A. N-(Phosphonacetyl)-L-aspartic acid (PALA)

This rationally synthesized pyrimidine antagonist was shown to be highly active against Lewis lung carcinoma. Early treatment proved to be curative and delayed treatment resulted in profound inhibition of tumor growth and increases in survival time. B16 melanoma implanted ip was also responsive to PALA. No activity was seen in a number of other experimental tumor systems including L1210 leukemia, glioma 26 and Ridgway osteogenic sarcoma. Current studies are directed toward determining why some solid tumors are sensitive to inhibition of de novo pyrimidine biosynthesis by this agent.

B. Maytansine

Following demonstration of the stathmokinetic effect of maytansine in tumor cell culture by Wolpert-DePhillipes et al (Biochem Pharmacol 24: 751, 1975) we evaluated the stathmokinetic effect of this compound on tumors in vivo. Maytansine produced a much greater and more prolonged metaphase arrest of P388 and L1210 leukemia cells in vivo than did the Vinca alkaloids. An LD_{10} dose of maytansine had about the same stathmokinetic effect as a 0.1 LD_{10} dose.

C. Methanol extract of Ervatamia hyneana

This crude plant extract, previously shown to be effective against P388 leukemia but not L1210 leukemia, was shown to have activity in ip B16 melanoma as well. The extract inhibits thymidine and uridine incorporation into tumor cells in vitro. Isolation and chemical characterization of the active principle is being carried out.

D. Inhibitors of protein biosynthesis

A number of drugs which inhibit protein biosynthesis at the ribosomal level in mammalian cells have activity in experimental tumor systems (i.e., emetine, anguidine, bruceantin). The classical inhibitors of protein synthesis, puromycin and cycloheximide, on the other hand, are inactive as antitumor agents. A study correl-

ating the biochemical effects of these agents with their cytotoxic properties was initiated using P388 leukemia cells. Of three glutarimide antibiotics, cycloheximide, acetoxycycloheximide, and streptovitacin A, only the latter compound has demonstrable activity in vivo against P388 leukemia. All three agents can completely inhibit protein biosynthesis in P388 cells but the inhibition by streptovitacin A was less reversible than the effects of the other two agents.

III. Resistance and Cross-Resistance to Antitumor Agents

A. Characteristics of sublines of P388 leukemia resistant to adriamycin and daunomycin

A subline of P388 resistant to adriamycin had a peculiar pattern of cross-resistance. P388/ADR was cross-resistant to all agents which bind to DNA, to mitotic spindle poisons, to all protein synthesis inhibitors except anguidine and to a number of miscellaneous agents. P388/ADR retained sensitivity to all antimetabolites and alkylating agents tested. Using labelled daunomycin and labelled actinomycin D, the mechanism of resistance was studied in vitro. It was shown that the rate of uptake was somewhat diminished but the rate of efflux from resistant cells preloaded with either of the labelled compounds was much greater than from sensitive cells.

B. Characteristics of a subline of P388 leukemia resistant to vincristine

Cross-resistance between vincristine and anthracyclines has been shown repeatedly in resistant tumor cells. Using a P388/VCR obtained from Dr. Richard Adamson we evaluated a number of drugs previously shown to be resistant in P388/ADR. The patterns of cross-resistance were not identical. P388/VCR retained sensitivity to ellipticine and AMSA, two DNA binders to which P388/ADR is completely cross-resistant. P388/VCR was similar to P388/ADR in that it was cross-resistant to protein synthesis inhibitors and all mitotic spindle poisons and was sensitive to alkylating agents.

IV. Development of New Tumor Models for Evaluation of Potential Antineoplastic Agents

Chemotherapy of human colon carcinoma xenografts in athymic nude mice

A program was initiated to evaluate some of the more promising agents in development in the DCT program against human colon carcinomas growing in athymic nude mice. Four human colon carcinomas were established in nude mice. Chemotherapy studies were successfully performed in two of the tumors, one poorly differentiated adenocarcinoma and one well differentiated. Both tumors had about a 6-day mass doubling time and drug treatment was delayed until tumors were about 200 mg in mass. MeCCNU was effective in inducing regressions in both tumors but only at rela-

tively toxic doses. PALA, AMSA and maytansine were inactive in both systems. Chlorozotocin was effective in one of the two tumors.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, this project is designed to develop fundamental information on the principles of effective chemotherapy. Studies are initiated in response to current clinical needs. In addition, fundamental studies on animal tumor models are directly geared to the goals of the Drug Research and Development program of the DCT for improved screening and detailed evaluation of potential antineoplastic agents and combination regimens.

Proposed Course of Project

During the next year efforts will continue on each of the projects described above. Increased emphasis will be placed on studies of the biochemical pharmacology of PALA, the methanol extract of Ervatamia hyneana, and cytotoxic inhibitors of protein biosynthesis. Experimental chemotherapy studies of actinomycins will be expanded to include a number of tumor systems unresponsive to actinomycin D. Comparative studies of anthracyclines will also be expanded to include a broad spectrum of naturally-occurring and semisynthetic structures within this class of compounds. Chemotherapy studies in human colon xenografts will be extended to include two more tumors and additional agents being developed toward clinical trial within the DCT program.

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2. Bessman, J. D., Johnson, R. K., and Goldin, A.: Permeability of normal and cancerous rat bladder to antineoplastic agents. Urology 6: 187-193, 1975.

3. Cohen, S. C., Gabelnick, H. S., Johnson, R. K., and Goldin, A.: Effects of cyclophosphamide and adriamycin on the healing of surgical wounds in mice. Cancer 36: 1277-1281, 1975.

4. Cohen, S. C., Gabelnick, H. L., Johnson, R. K., and Goldin, A.: Effects of antineoplastic agents on wound healing. Surgery 78: 238-244, 1975.

5. Goldin, A., and Johnson, R. K.: Antitumor effect of adriamycin in comparison with related drugs and in combination chemotherapy. In Adriamycin Review (Staquet, N., Tagnon, H., Kenis, Y., et al, Eds.) European Press Medikon, Ghent 37-45, 1975.

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Appendix A

Tumor Biology Section
Contracts

Contract # NO1-CM-53767

Principal Investigator: Dr. Roger N. Rosenberg

Co-Principal Investigator: Dr. Fred Baskin

University of Texas Health Science Center at Dallas

$59,975

Objectives:

To develop tumor cell sublines resistant to drugs of interest to the National Cancer Institute, determine patterns of cross-resistance among established and potential antineoplastic agents, and evaluate the mechanism of resistance to selected agents.

Major Findings:

Mouse C1300 neuroblastoma was exposed to twelve drugs in tissue culture to obtain resistant sublines. The 12 drugs are: vincristine, maytansine, BCNU, chartreusin, anguidine, Bakers antifol, activated cyclophosphamide, bleomycin, L-alanosine, ICRF-187, adriamycin and AT-125 (NSC 163501). A subline 50-fold resistant to maytansine was developed which proved to be equally cross-resistant to other mitotic spindle poisons, vincristine and colchicine. 30-fold cross-resistance to adriamycin was also observed. Relative sensitivity to ICRF-187 chartreusin and actinomycin D was retained. Sublines resistant to Baker's antifol and vincristine were also obtained.

Significance to Biomedical Research and Program of the Institute:

This contract, in supplying sublines with defined mechanisms of resistance and patterns of cross-resistance, will provide tools for determining whether chemical analogues of established drugs or unique structures are, in fact, biochemical analogues. In addition this contract will provide basic information on the cytotoxic activity and mechanism of action of unique antineoplastic agents.

Proposed Course of Project:

Efforts will continue to develop resistant sublines to all of the drugs listed above, to determine the mechanism of resistance in established sublines and to determine the patterns of cross-resistance to a broad spectrum of cytotoxic agents.

Contract # NO1-CM-57030

Principal Investigator: Dr. Arthur E. Bogden

Co-Principal Investigator: Dr. Diane Kelton

Mason Research Institute

$142,580

Objectives:

To develop new in vivo tumor systems with potential predictive value in selecting drugs which will be active in human solid tumors, to evaluate growth characteristics, cytokinetic parameters and chemotherapeutic response to potential and established antitumor agents of transplantable, spontaneous, viral-induced or carcinogen-induced animal tumors with special emphasis on human tumor xenografts in nude mice.

Major Findings:

Facilities for and a colony of athymic nude mice were established. A number of human solid tumors from tissue culture and primary explants were established in nude mice. The biology and chemotherapeutic response of two new transplantable mouse tumors were determined. These tumors are histologic types that have not been well studied: a transitional cell bladder carcinoma (FCB) and an ovarian carcinoma (M5076).

Significance to Biomedical Research and the Program of the Institute:

Experimental tumor systems currently in use do not select, with any degree of precision, agents which will be effective in the treatment of common human solid tumors. It would be beneficial to have a spectrum of experimental solid tumors which more closely resemble human carcinomas in biology and chemotherapeutic response. This contract is designed to develop such tumor systems.

Proposed Course of Project:

A number of human tumor types, particularly mammary adenocarcinoma, will be established as serially transplantable lines in nude mice. During the coming year there will be an emphasis on chemotherapy of human tumors with experimental and established antineoplastic agents. As the colony of nude mice increases there will be a de-emphasis of studies of transplantable murine tumors.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-03577-03 LEC

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunochemotherapy Studies in Various Tumor Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David P. Houchens	Senior Staff Fellow	LEC NCI
OTHERS:	Abraham Goldin	Actg. Chief, Lab. Exptl. Chemot.	LEC NCI
	Angela Santoni	Visiting Fellow	LEC NCI
	Rainhardt Osieka	Guest Worker	LEC NCI
	Randall K. Johnson	Actg. Head, Tumor Biology Sect.	LEC NCI

COOPERATING UNITS (if any) F. Campanile, L. Crino, E. Bonmassar, Univ. of Perugia, Perugia, Italy; B. Giovanella, Stehlin Cancer Foundation; M. Kende, T. Aoki, S. Rosenberg, NCI; A. Ovejera, Battelle Memorial Institute; T. Marks, RTI, Research Triangle Park; A. Ommaya, NINCDS; L. Cahan, Dept. of Neurosurgery, Univ. of Calif. Los Angeles; L. Albright, Dept. of Neurosurgery, Univ. of Pittsburgh; A. Daniel, FDA.

LAB/BRANCH

Laboratory of Experimental Chemotherapy

SECTION

Immunochemotherapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to develop models for investigations of interactions between antitumor agents and the host's immune response and the application of immunotherapy.

The major areas of immunotherapy in this project are the use of antigenically altered tumor cells, non-specific immune stimulation and the use of athymic (nude) mice to develop therapy models of human tumors.

FORM 6040
(1-76)

Project Description:

Objectives:

I. To study the antigenic changes occuring in tumors following treatment with antineoplastic agents *in vivo*.

II. To develop a program employing athymic (nude) mice for the growth and treatment of human tumors.

III. To develop appropriate models for investigation of interactions between antitumor agents and the host's immune response and the application of immunotherapy.

Methods Employed: Standard laboratory procedures were employed.

Major Findings:

I. Studies on Drug Induced Antigenic Changes of Tumor Cells.

A. *In vivo* studies (D. Houcheas, E. Bonmassar, M. Kende, A. Goldin)

Lymphomas induced by Moloney and Rauscher leukemia viruses were used to determine whether antigenically altered tumors induced by DIC would retain their original properties and/or have new antigenic properties. Syngeneic mice could be protected against challenge with the parental tumor by presensitization with the drug-altered sublines while unrelated tumor lines were incapable of protecting them. The DIC altered subline of LSTRA was used for treatment of the LSTRA in conjunction with chemotherapy (BCNU) and this immunochemotherapeutic regimen produced significant increases in number of survivors and increases in median survival time compared to either treatment alone. Tolerance studies indicated that there are novel antigens and parental tumor antigens associated with the drug treated sublines.

B. *In vitro* studies (A. Santoni, T. Campanile, E. Bonmassar, A. Goldin and D. Houcheas)

Cell mediated cytotoxic response *in vitro* to the DIC altered LSTRA tumor line (LD-1) was investigated using mixed leukocyte tumor cell cultures (MLTC). The responding cells were either spleens from normal BALB/c mice (primary MLTC) or spleens from BALB/c mice that rejected the LD-1 tumor (secondary MLTC). Irradiated LD-1 and LSTRA cells were used as syngeneic stimulators.

As assessed by short term ^{51}Cr release assay, cytolytic activity was not generated in primary MLTC. High cytolytic activity specific against LD-1 was generated in secondary MLTC. Little or no response was obtained against LSTRA. In kinetics studies, optimal cytotoxic activity was seen in MLTC on incubation for 5-7 days.

The ability of spleen cells to mount a secondary cytolytic response in vitro could be observed as late as 4 months following primary in vivo immunizations.

II. Nude Mouse Studies

A. Drug toxicity in Nude Mice (D. Houchens, R. Johnson, R. Marks)

Athymic (nude) mice are being used in increasing frequency for chemotherapy studies of human tumors. There has been no reported toxicity data for tumor chemotherapeutic agents in this strain of mice. Therefore eleven anticancer agents were tested in nude mice on an NIH Swiss background and on age and sex matched Swiss control mice. The LD_{10} and LD_{50} doses, as determined by probit analysis, were higher in nudes than in Swiss mice for BCNU, methotrexate, bleomycin, 5-FU, vinblastine, vincristine and actinomycin D. The toxic doses were the same in the two strains for cyclophosphamide while DTIC was the only drug which was more toxic in nudes than in Swiss mice. Adriamycin and melphalan were not tested in Swiss mice but the nudes were able to tolerate the drug at the same doses as F_1 hybrid mice as reported in the literature. The results indicate the need to conduct toxicity studies in nude mice in order to determine the treatment schedule with various drugs.

B. Human Tumor Growth in Nude Mice. (K. Osieka, D. Houchens, R. Johnson, B. Giovanella, S. Rosenberg, A. Goldin)

As reported in this section last year, a program for chemotherapy and immunotherapy in nude mice has been developed. The laboratory for this study has been completed with facilities for holding and testing 600 mice. Presently two human colon tumors supplied by Dr. Giovanella are being grown and various chemotherapeutic agents are being tested on them. For results of these studies see the Tumor Biology Section Report (#ZO1-CM-07100-03). Dr. Rosenberg is supplying surgical specimens from osteosarcoma patients. One is presently implanted and growing.

C. Hemopoietic-histocompatibility (Hh) studies in nude mice. (D. Houchens, E. Bonmassar, F. Campanile, L. Crino, A. Goldin)

Intact or lethally irradiated nude mice on a BALB/c background were inoculated with an H-2 and Hh incompatible lymphoma to determine the effect of the anti-Hh allograft response.

The tumor growth was markedly inhibited in the nude mice while it grew in syngeneic control mice.

The lack of tumor growth in the lethally irradiated nude mice excludes the possibility of a conventional allograft reaction being responsible but strongly suggests a highly efficient anti-Hh allograft reaction in these mice. Murine glioma and melanoma cells, used as controls, grew in the nude mice thus confirming the obser-

vation that Hh antigens are restricted to hemopoietic or lymphoma tissues.

III. Immunochemotherapy

A. Non-specific Stimulation of the Immune System

1. Corynebacterium parvum plus adriamycin (D. Houchens, R. Johnson, A. Ovejera, A. Goldin)

Corynebacterium parvum was employed in antitumor tests against four murine tumor models in $B6D2F_1$ mice. The C. parvum was effective at all doses and schedules tested against P388 leukemia, B16 melanoma and Lewis lung carcinoma but was ineffective against L1210 leukemia. Combination immunochemotherapy of P388 leukemia and Lewis lung carcinoma with C. parvum and adriamycin was better than either regimen alone in increasing life span of mice with tumors.

The antitumor effects of C. parvum were found to be due to non-specific stimulation of the host rather than direct cytotoxic action on tumor cells. C. parvum protected the mice when given before as well as after tumor challenge. In vitro ^{51}Cr release assay showed that the peritoneal cells were cytotoxic to P388 tumor cells but spleen cells were not. While the C. parvum was effective against P388 in conventional mice, it was ineffective on P388 growing in athymic (nude) mice. Thus the antitumor effect in this tumor system is T-cell dependent.

2. OK-432 plus chemotherapy (D. Houchens, T. Aoki, A. Goldin)

Experiments were initiated based on observation by Dr. Aoki that the Japanese compound OK-432 (Picibinal) increased the life span of AKR mice with spontaneous leukemia. Protocols using combinations of cyclophosphamide or 5-FU plus OK-432 have been started. Because of the long term of such experiments (> 6 months), data is inconclusive at this point. Dr. Aoki's laboratory is testing sera samples from those mice for cytotoxic response and for presence of virus.

B. Immunotherapy Studies in Murine Glioma Model. (D. Houchens, L. Cahan, L. Albright, A. Daniel, A. Ommaya)

Collaborative studies on murine and human glioma were completed and a manuscript has been submitted. Cell membranes from malignant human and murine gliomas were treated with lithium diodosalicylate (LIS) and the LIS extracted proteins were separated by electrophoresis. Human glioma extracts contained proteins which differed in both the number of bands and in their gel locations when compared to other glioblastoma extracts or to autologous normal brain extracts. Glycoproteins were extracted from four of the ten human

malignant glioma specimens and none exhibited electrophoretic or immunodiffusion similarities with carcinoembryonic antigen (CEA).

Cell membrane-enriched fractions from a murine glioma were successful in immunizing mice against subsequent intracranial tumor challenge. Glycoproteins extracted from these membranes by the LIS procedure failed to afford this protection.

Significance to Biomedical Research and the Program of the Institute:

The studies are part of a program to set up new strategies for the immunochemotherapy of malignant tumors. Attempts are being made to develop models suitable for studies of immunodepression induced by drug treatment and by tumor-associated factors in tumor-bearing hosts. Particularly a novel approach is being employed in an attempt to obtain new antigenic targets at the level of tumor cells through antigenic changes induced by drug treatment in animals transplanted with neoplastic cells.

Proposed Course of Project:

The Immunochemotherapy Section will be reorganized during the coming year with the employment of a new professional staff. The overall course will continue in the development of animal models for immunochemotherapy regimens.

Publications

1. Bonmassar, E., Campanile, F., Houchens, D., Crino, L., and Goldin, A.: Impaired growth of a radiation-induced lymphoma in intact or lethally irradiated allogeneic athymic (nude) mice. Transplantation 20: 343-346, 1975.

2. Bonmassar, E., Houchens, D. P., Fioretti, M., and Goldin, A.: The uptake of 5-iododeoxyuridine as a measure of tumor growth and tumor inhibition. Chemotherapy 21: 321-329, 1975.

3. Campanile, F., Houchens, D. P., Gaston, M. R., Goldin, A., and Bonmassar, E.: Increased immunogenicity of two lymphoma lines following drug treatment in athymic (nude) mice. J. Nat. Cancer Inst 55: 207-209, 1975.

4. Goldin, A. and Houchens, D. P.: Preclinical approaches in tumor immunochemotherapy. Monograph, Brussels, (in press), 1976.

5. Houchens, D. P., Bonmassar, E., Gaston, M. R., Kende, M. and Goldin, A.: Drug-mediated immunogenic changes of virus-induced leukemia cells in vivo. Cancer Res, (in press), 1976.

6. Houchens, D. P. and Goldin, A.: The combined modality of chemotherapy and immunotherapy in treatment of experimental tumors. 2nd Oncology Congress, Rumania (in press).

7. Houchens, D. P., Iorio, A., Barzi, A., Goldin, A. and Bonmassar, E.: Inhibition of antilymphoma allograft response in normal and lethally irradiated mice by cyclophosphamide (NSC-26271) and Isophosphamide (NSC-109274). Cancer Chemother Rep 59: 967-973, 1975.

8. Johnson, R. K., Houchens, D. P., Gaston, M. R., and Goldin, A.: Effects of levamisole (NSC-177023) and tetramisole (NSC-102063) in experimental tumor systems. Cancer Chemother Rep 59: 697-705, 1975.

9. Ward, J. M., Houchens, D. P., Collins, M. J. Jr., Young, D. M. and Reagan, R. L.: Naturally occuring sendai virus infection of athymic nude mice. Vet. Pathol. (in press).

Appendix A

Immunochemotherapy Section
Contracts

Contract # NO1-CM-57041

Principal Investigator: Dr. Michael Sigel

University of Miami Medical School

$74,209

Objectives:

To evaluate the immunosuppressive activity of antineoplastic agents in order to aid in the selection of candidate drugs. To include comparative studies on the humoral and cell-mediated immune responses.

Major Findings:

I. Effect of antitumor drugs on humoral immune responses:

Cyclophosphamide, melphalan and 3-deazauridine were assayed by the plaque-forming cell (PFC) technique for immune response to sheep erythrocytes. All three drugs drastically reduced the PFC responses when given after the antigens. Cyclophosphamide and melphalan were also given before the antigen and both produced marked suppression. All three drugs also caused marked reduction in leukocyte population.

II. Effects of anticancer drugs on cell-mediated responses:

The immunologic competence of lymphocytes was assessed following treatment of animals with cyclophosphamide, actinomycin D, hexamethylmelamine, Me-CCNU, and methotrexate. One injection of cyclophosphamide reduced concanavalin A response and it remained significantly reduced as long as 16 days later. The other 4 drugs marked reduced the Con A response for 5 days and the response had returned to normal by 12 days.

Significance to Biomedical Research and the Program of the Institute:

The immunodepressive activity of the majority of antitumor compounds has negative consequences on the host's antimicrobial and antiviral defenses and on potential synergistic effects which may occur between chemotherapy and the host's antitumor responses. These studies can help to elucidate the effects of these drugs on the various portions of the immune system to select the ideal treatment schedules with the least adverse effects.

Proposed Course of Project:

To continue assaying drugs which are of present clinical interest and to add new assay systems as they are developed for optimal response.

Contract # NO1-CM-57039

Principal Investigator: Dr. Enrico Mihich

Health Research, Inc., New York

Roswell Park Memorial Institute

$86,380

Objectives:

To evaluate the immunosuppressive activity of antineoplastic agents in order to aid in the selection of candidate drugs. To include comparative studies on the humoral and cell-mediated immune responses.

Major Findings:

The following drugs were sent to the contractor for testing:

Vincristine	BCNU
Vinblastine	Methyl CCNU
Vindesine	PCNU
Chlorozotocin	5-Fluorouracil
Streptozotocin	Ftorafur
Maytansine	Adriamycin
Cytosine Arabinoside	Daunorubicin

I. In vivo studies

A. Kinetics of immune responses:

P815 tumor was used to measure the complement dependent cellular cytotoxicity (CDCC) and complement independent cellular cytotoxicity (CICC) The CDCC response was maximum at 5 days then decreased. The CICC response was high at 7 days and gradually increased to the highest point on day 21.

B. Irradiated Immunogens:

X-irradiation killed P815 cells were also used as the immunogen. One injection gave no measurable CDCC but there was a weak CICC response. Multiple injections produced good responses.

C. Drug Treatment:

The P815 system was used for drug testing. Adriamycin and daunorubicin were tested on day 0 or +2 in relation to the antigens. At equimolar doses, adriamycin was less suppressive than daunorubicin. In some test adriamycin was found to be immuno-stimulatory.

Maytansine was suppressive on testing by CDCC and vindesine was suppressive to both CDCC and CICC when given before, on the same day or after the antigen.

II. In vitro studies:

P815 was used to stimulate C57BL/6 spleen cells in culture. This system was used in order that the effects of drugs on more easily identifiable cells and stages of immune response development could be ascertained. Drugs could be used to either pre-test the donors or could be used directly in the culture. A single treatment of spleen donor mice with either adriamycin or daunorubicin greatly enhanced the CICC while cytosine arabinoside had no such effect. The tests thus far indicate that more than one cell population may be required to develop CICC in vitro.

Significance to Biomedical Research and the Program of the Institute

The immunodepressive activity of the majority of antitumor compounds has negative consequences on the host's antimicrobial and antiviral defenses and on potential synergistic effects which may occur between chemotherapy and the host's antitumor responses. These studies can help to elucidate the effects of these drugs on the various portions of the immune system to select the ideal treatment schedules with the least adverse effects.

Proposed Course of Project:

To continue assaying drugs which are of present clinical interest and to add new assay systems as they are developed for optimal response.

Contract # NO1-CM-53766

Principal Investigator: Dr. Gustavo Cudkowicz

Research Foundation of State University of New York

$134,301

Objectives:

The objective of this project is to perform in vivo and in vitro basic investigation and development of new experimental models for studying the effects of anticancer agents on immune responses. Specific aims include: 1) evaluation of the efforts of selected agents on T and B cells, on macrophages and on the immune responses against Hh; 2) development of new experimental models for evaluating the effects of anticancer agents on the different components of the immune system.

Major Findings:

The major results in this study have involved the development of systems that will be used to assay drugs.

A. Induction of Cytotoxic Effector Cells in vitro:

Results have shown that cell-mediated cytotoxicity to Hh alloantigens is selectively dependent on monocyte-macrophages like cells sensitive to silica particles and/or to rabbit anti-mouse bone marrow serum, whereas CMC to H-2 alloantigens is independent of such cells. The cytotoxicity assay offers the opportunity to expose to drugs prekiller cells capable of responding to different types of antigens, accessory cells required for certain CMC responses, and functional effector cells after induction.

B. Helper function for antibody formation in vivo:

Results showed that: 1) Low dose antigen stimulation is optimal for amplifier cells but not for helper cells. Certain chemical modifications of complex natural antigens increase the immungenicity for amplifier cells, but not that for helper cells; 2) Activation of amplifier cells results in increased production of IgG but not of IgM antibody; 3) An in vivo system was developed in which amplifier and helper cells are harvested from separate mice (so that each cell type can be exposed to drugs in the absence of the other), mixed in vitro and transferred with B cells into irradiated test recipients. By exploiting the differential sensitivity of two kinds of T cells to antiserum and to modified antigens, it was possible to obtain synergism in the generation of IgG antihapten antibody-forming cells.

Significance to Biomedical Research and the Program of the Institute:

The systems thus far developed have demonstrated the ability to selectively predict the effect of agents on selected cell populations in the immune system.

Proposed Course of Project:

During the coming contract year, pilot drug studies will be employed in these and other systems to further elucidate effects on cellular and humoral components.

Contract # NO1-CM-53826

Principal Investigator: Dr. Federico Spreafico

Istituto di Ricerche Farmacologiche

"Mario Negri"

$77,175

Objectives:

This contract is to 1) develop new tumor-host models using spontaneous or induced tumors in inbred or genetically defined F_1 hybrids; 2) test combined modalities, of immunotherapy and chemotherapy using active, adaptive, passive or non-specific immunotherapy.

Major Findings:

A. Combined chemoimmunotherapy with non-specific immunoadjuvants:

Corynebacterium parvum was used in combination with adriamycin for treatment of L1210 leukemia, LSTRA leukemia, Lewis lung carcinoma. The combination treatment was significantly better than either regimen alone in increasing the life span.

B. Chemotherapy combined with active immunotherapy using immunogenic drug-treated tumor sublines:

The L1210 and LSTRA tumor systems were employed to study the effects of combination therapy, employing BCNU and DIC altered tumor cells. The results indicate immunogenic sublines can be effectively used in therapy experiments.

C. Other types of chemoimmunotherapy:

Other systems have been developed and are being explored for further studies. These include: 1) Immunochemotherapy with antitumor agents and immune responses of the Hh type; and 2) Immunochemotherapy with chemoresistant lymphocytes. Both of these systems have shown promising results.

Significance to Biomedical Research and to the Program of the Institute:

The approaches used will aid in the ultimated development of predictive models and treatments for the combined immunochemotherapy of human tumors.

Proposed Course of Project:

Further development of the system is planned with additional use of drugs of clinical significance.

ANNUAL REPORT

LABORATORY OF MEDICINAL CHEMISTRY AND BIOLOGY

DRUG RESEARCH AND DEVELOPMENT PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Laboratory of Medicinal Chemistry and Biology is a new organizational entity resulting from the consolidation of previously established sections from within the Drug Development and Drug Evaluation Branches of the Drug Research and Development Program, DCT: the Laboratory of Chemical Pharmacology, Experimental Therapeutics Program DCT: and the Laboratory of Biochemistry, DCBD. The aim in establishing the new Laboratory has been to bring about a closer relationship between medicinal chemists involved in the design and synthesis of new antitumor agents, and biologists involved in the elucidation of their biochemical pharmacology and in their clinical evaluation. It was felt in proposing this new organizational structure that knowledge of the molecular biology and etiology of cancer is approaching the level at which the biologist can now make useful early contributions to the rational design of new antitumor agents, rather than being restricted almost entirely to biochemical, pharmacological and clinical studies on agents whose anti-neoplastic activity has already been established on an empirical basis. While it is recognized that it would be premature and over-optimistic to propose that empirically-based screening approaches can in the foreseeable future be abondoned in favor of specific, rational antitumor drug design, the time now appears to have arrived when a mixture of empirical and rational approaches is warranted. Examples of drug classes in which opportunities for specific, target-directed design are now possible are agents affecting structure and function of tumor cell surface glycoproteins; inhibitors of viral oncogenesis; agents with specific or unique DNA-binding properties; transition-state inhibitors for key enzyme sites; and agents with specific tissue-localizing properties. In these and a number of other rapidly expanding research areas, the state of the art appears sufficiently advanced that the biologist with specific expertise can make contributions of value to the medicinal chemist in the design of new antitumor agents and in the modification of existing agents.

Conversely, from the point of view of the medicinal chemist, a closer inter-relationship with the molecular biologist and the clinician offers the opportunity of obtaining specific biological assay data with the minimum of delay, in order to guide the course of further synthesis. In addition to providing routine antitumor screening, the biologist can design assay methods tailored to the specific target site under consideration. Assays for parameters such as inhibition of viral replication, immunostimulation, enzyme inhibition or tumor cell membrane binding can be provided where relevant to the activity under consideration. Thus, the juxtaposition within a single laboratory of medicinal chemists and biologists from a variety of different disciplines offers the prospect of accelerating drug development, and of

supplementing the traditional empirical approaches which have yielded most of our clinically successful antitumor drugs up to the present time with specific target-site directed drug design programs.

The major organizational entities within the new Laboratory of Medicinal Chemistry and Biology, in addition to the Drug Design and Chemistry Section, are the Molecular Biology and Methods Development Section, with the capacity to assess the effects of new agents on cell growth and cell cycle progression; a membrane biology group, with a specific interest in the role of tumor cell membrane alterations in drug resistance; a biochemical pharmacology section concerned with the mode of action of antitumor agents at the molecular level; and an applied pharmacology section with the capacity to carry out drug disposition and metabolic studies in experimental animals and at the Phase I level.

Some of the individual research projects presently under way in the Laboratory are summarized below.

A major long-term interest of the Drug Design and Chemistry Section of this Laboratory has been the development of agents with activity against primary and metastatic tumors of the central nervous system. While limited success has been obtained in some selected areas (e.g., the treatment or prevention of meningeal leukemia with intrathecal methotrexate), little overall progress has been made in the treatment of CNS neoplastic disease. While a number of reasons can be advanced for the refractoriness of these tumors to chemotherapy, a common factor is the difficulty in obtaining adequate drug concentrations at the tumor site. For this reason, emphasis has been placed in the Laboratory in modifying the physical properties and transport characteristics of established antitumor agents in order to obtain compounds with optimal ability to penetrate the anatomical barriers of the central nervous system in sufficient concentrations to be effective at the tumor site. Particularly promising results have been obtained in model systems with the diaminodiaziridinylbenzoquinone family of compounds. The objective is the preparation of compounds possessing both murine CNS activity and adequate water solubility. Nineteen compounds were synthesized. Eight of these compounds gave 60 day survivors in the intracerebral (IC) ependymoblastoma tumor model in more than one experiment. ILS values in excess of 200% were observed in each of these experiments. Five compounds reproducibly gave intraperitoneal (IP) L1210 activity of ILS > 100% with long term (30 day) survivors. Five showed substantial (ILS reproducibly > 50%) IC L1210 activity. As would be expected from the L1210 results, 30 day survivors were seen in the IP P388 test system. Four members of this family were reproducibly active in the B16 melanoma system. Optimum doses ranged from 0.1 to 25 mg/kg while water solubilities ranged between 60 and < 0.1 mg/ml. One of the most active compounds was a urethane quinone derivative (NSC 182986) which had a water solubility of ca. 0.5 mg/ml and the following activities (tumor; ILS): IP L1210, 169%; IC L1210, 84%; IP P388, 138%; IC P388, 51%; IP B16, 70%; IC ependymoblastoma, 348% (four of six 99 day survivors). Redox potentials and partition coefficients are being measured in this series in order to attempt a Hansch-type quantitative structure-activity analysis.

Another approach which this laboratory has applied to the development of CNS-

active antitumor agents is the modification of compounds known from studies in other areas of pharmacology to enter the central nervous system readily by the addition of alkylating or other specific groups. Psychoactive compounds such as the phenothiazines, barbiturates and hydantoins have been utilized as potential carriers for such groups. This project has led to the synthesis of spirohydantoin mustard (NSC-172112), a compound recently selected on the basis of its excellent murine intracerebral ependymoblastoma and intraperitoneal P388 activity for animal toxicology studies and possible clinical trial.

Another major interest of this laboratory has been the modification of the antitumor agent 5-azacytidine, a compound which is of proven value in the treatment of acute myelocytic leukemia, but which has the major practical disadvantage of being highly unstable in aqueous solution, undergoing decomposition in a matter of hours to cleavage products of unknown antitumor activity and toxicity. The most promising candidate to emerge from these studies is 5,6-dihydro-5-azacytidine, prepared by the chemical reduction of 5-azacytidine. This compound has been completely characterized both as the hydrochloride salt (NSC 264880) and the free base (NSC 265483) by gas chromatography, mass spectrometry, N.M.R., U.V. and I.R. spectroscopy and elemental analysis. Acid hydrolysis removed the sugar and produced 5,6-dihydro-5-azacytosine hydrochloride. This compound (NSC 265485) was identical with that produced by the reduction of 5-azacytosine. While solutions of 5-azacytidine in water undergo 25-40% decomposition after 24 hours at room temperature, dihydro-5-azacytidine hydrochloride appears to undergo no decomposition under similar conditions over a period of 60 days.

Limited antitumor activity data for 5,6-dihydro-5-azacytidine are available at the present time. Two separate in vivo L1210 tests (Q4D schedule) have been completed. In both experiments, maximum activity was obtained at the top dose tested (400 mg/kg, ILS 62% and 600 mg/kg, ILS 104%). U.V. analysis shows less than 0.06% of 5-azacytidine in our samples of dihydro-5-azacytidine. 5-Azacytidine run in parallel experiments gave ILS values of 144 and 138% at 10 and 20 mg/kg respectively. 5,6-Dihydro-5-azacytidine had a therapeutic index (highest active dose divided by lowest active dose) of 50 in these Q4D experiments and has a water solubility greater than 200 mg/ml. The optimum treatment schedule for this agent is presently being established. In vivo tests are then planned in the B16 melanoma, P388 leukemia and the 5-azacytidine resistant P388 tumor systems as well as other appropriate tumor models. Additional synthetic work is in progress in the area of reduced nucleosides including tetrahydro-5-azacytidine and reduced Ara-C. It is also hoped that several new chemical reactions discovered during the course of the 5-azacytidine work will allow the synthesis of arabinosyl-5-azacytosine, which the literature indicates is not presently attainable.

In parallel biological studies, we have observed that 5,6-dihydro-5-azacytidine like 5-azacytidine, is a substrate for cytidine deaminase; it is more slowly inactivated by this enzyme than is the parent compound, however, a property which could be of advantage should the dihydro derivative reach clinical application.

Another approach presently being followed in the medicinal chemistry section is the development of compounds capable of generating small reactive molecules

in vivo. An example is the preparation of oxygen-substituted benzaldoximes as potential HCN generators. Structural modifications have been chosen to maximize the fragmentation pathway producing HCN at the expense of the organic nitrile pathway. The quantitative aspects of the reaction are being studied by gas chromatography and gc/mass spectrometry; and additional synthetic work is planned if these compounds show significant activity in in vitro and in vivo antitumor testing.

Considerable interest currently exists in this and other laboratories in modifying dosage forms of antitumor agents in order to alter, and possible enhance, their pharmacological activity. Approaches which have been proposed or attempted are incorporation of antineoplastic drugs in liposomes, binding to artificial polymers, complexing to site-directed molecules (e.g., antibodies, hormones), or to macromolecules such as DNA or serum proteins. Within this laboratory, forty antitumor drug-protein complexes utilizing various combinations of 5-azacytidine, ara-C, actinomycin D, an active acridine derivative, cytembena, dichloro-MTX, ellipticine and AT-125 with albumin, fibrinogen and chemically modified albumin were prepared and evaluated in the L1210 or P388 systems. Covalent as well as ionic or charge-transfer complexes were prepared. The parent compound was routinely evaluated in the same experiment with the complex. Increases in ILS values with the complexes relative to the uncomplexed drug were observed for several of these combinations. Also observed was (1) an increase in solution stability (5-azacytidine) and (2) an apparent increase in water solubility (acridine NSC 141549). Studies are continuing in this area.

A long-term interest of this laboratory is the comparative evaluation of techniques for the development of quantitative correlations between antitumor activity and structural and physico-chemical features of antitumor drugs. The potential use of such correlations is to generate leads for new synthesis, to increase the rationale for selection of new compounds to be tested, and to reduce the number of synthetic analogs necessary to obtain the optimally active member of a new series. Computer techniques are used to analyze and organize biological screening data. These data are then correlated with molecular structural parameters using computerized regression analysis techniques. Such studies are now in progress with the anthracycline (adriamycin) antibiotics, the terephthalanilide series, and the Cain acridine series.

A recent development of great interest to this laboratory has been the development within the pharmaceutical industry of several classes of potent inhibitors of the mammalian enzyme adenosine deaminase. A large number of presently available adenosine analogs exhibit antitumor, antiviral and antibacterial activity in in vitro model systems, but their usefulness in vivo has been limited by rapid enzymic deamination and consequent inactivation. The co-administration of an effective adenosine deaminase inhibitor would offer the possibility of preventing this inactivation and thus of potentiating the activity of these analogs. Studies have been initiated within this laboratory with the combination of the adenosine analog cordycepin (3'-deoxyadenosine) and the adenosine deaminase inhibitor 2'-deoxycoformycin; this project is being carried out jointly with the Laboratory of Chemical Pharmacology, ETP, DCT. 2'-Deoxycoformycin has been found to be extremely effective

in potentiating the activity of cordycepin both in cell culture systems (P388, L1210, and the human leukemia cell line CEM), and in experimental tumor systems in vivo. Studies are presently being extended to determine whether 2'-deoxycoformycin exerts a similar synergistic effect with other adenosine analogs.

Studies are continuing on the mode of action of the ansa macrolide maytansine, a plant product which was isolated and characterized by Kupchan and his co-workers in 1972, and which is now in early clinical trial. In studies recently completed in this laboratory, maytansine was found to impair microtubule formation in mammalian cells and to produce metaphase arrest similar to that seen with the Vinca alkaloids. Topics of present investigation are the nature of the maytansine binding site, electron microscopic studies of the alterations in microtubular structure produced by the drug, and the development of bioassay methods for maytansine. It has been found by electron microscope (EM) studies that, in L1210 cells grown in suspension culture and treated with maytansine, there is virtually a complete loss of all microtubules. In untreated mitotic cells, microtubules are well formed and well delineated. It has also been found by EM analysis that the antimitotic effects of maytansine are similar to those seen in parallel cultures treated with colcemid, vincristine sulfate and griseofulvin, although maytansine is more potent.

In studies with microtubular protein from mammalian brain, we have found that maytansine competes for the same binding site as that utilized by the Vinca alkaloids; the binding, however, is considerably tighter than that seen with the reference compound, vincristine. Studies are presently under way to delineate more clearly the mode of binding of maytansine to purified brain tubulin.

A long-term interest of this laboratory has been the pharmacology of the folate antagonists, and in particular, of the widely used agent methotrexate. With the recent introduction into clinical practice of high-dose methotrexate protocols, new problems have arisen, in particular renal toxicity, which were not observed with the lower-dose schedules previously employed. We have therefore carried out studies on the pharmacokinetics of high-dose methotrexate in patients receiving between 50 and 200 mg/kg. Peak plasma levels were proportional to dose and some 20 to 200-fold higher than that achieved with conventional doses. Peak urine levels were 10-fold greater than the peak plasma MTX levels and exceeded the maximum concentration of MTX soluble in urine at pH 6 or below. Animal studies were designed to test the hypothesis that limited solubility of MTX was responsible for the renal toxicity associated with the high-dose regimen. Histological sections and autoradiographs of tissue slices from the kidneys of monkeys which had received high-dose MTX, and which developed the syndrome of renal failure, showed crystalline-like deposits of MTX or MTX-derived material within the lumen of the renal tubules. Chromatographic studies on the urine from human subjects and Rhesus monkeys receiving high-dose MTX revealed the presence of a metabolite of MTX, 7-hydroxymethotrexate, which was not seen at lower dose levels. Although 7-hydroxymethotrexate is some two orders of magnitude less effective as an inhibitor of dihydrofolate reductase than MTX and does not contribute significantly to the folate antagonist activity of MTX, it may be of significant

renal toxicological importance because of its limited aqueous solubility.

A project has been initiated within the laboratory on the feasibility of nutritional and pharmacological intervention to promote the activity of established and potential cancer chemotherapeutic agents, and to increase their selectivity for tumor cells. Approaches to this goal include the following: 1) alteration of the nutritional status of tumor cells, both in culture and in vivo, to produce conditions interfering with the metabolic inactivation of the chemotherapeutic agent. This approach is particularly directed against resistant cell lines; 2) modification of the cell membrane of susceptible and resistant varieties of tumor cells by the use of either available or newly synthesized membrane-reactive agents; 3) synthesis of analogs of known agents in order to introduce modifications to make them more suitable for the regimens described above.

In initial studies, we have investigated the membrane-reactive agent amphotericin B, an antibiotic presently in clinical use as an antifungal agent. The selectivity of this agent is considered to be due to the presence of ergosterol in the membranes of suceptible fungi. We have investigated the effect of ergosterol added to the growth medium of L1210 cells on the subsequent sensitivity of these cells to amphotericin-B. Cells grown in ergosterol showed increased sensitivity to the lytic action of the antibiotic. Cholesterol at equivalent levels was ineffective and at a concentration of amphotericin-B which was not cytotoxic to cells grown in cholesterol, over a two-log kill was observed with cells grown in ergosterol.

In view of the continued and increasing clinical interest in melphalan (phenylalanine mustard; L-PAM), a project has been initiated on the relationship between the membrane transport and the cytotoxicity of this agent. The lethal action of melphalan on L1210 cells in vitro was shown to be determined by the amino acid content of the medium. Various amino acids protected the cells from the onset of cytotoxicity to different extents, and this protection was both concentration and time dependent. Present evidence suggests that the protection is mediated through competition with melphalan for a high affinity transport site. The amino acids affording protection against melphalan cytotoxicity do not correspond to those reported in the literature to be transported by a high affinity mechanism in other cell types. This suggests that the establishment of both an amino acid protection profile for tumor cells in vitro and another profile for protection against acute toxicity in the host, may permit the formulation of an amino acid mixture which when injected with melphalan into the tumor bearing animal would both protect the host and direct the agent to the tumor.

The Molecular Biology and Methods Development Section of the Laboratory of Medicinal Chemistry and Biology conducts both an intramural and a contract-supported research program whose objective is the characterization of the mode of action of new drugs at the molecular level, and the development of new systems for the detection of antitumor agents. Studies are not restricted to agents under development intramurally within the Laboratory of Medicinal Chemistry and Biology, but encompass agents being developed by the Drug Research and Development Program as a whole.

Within the intramural [illegible] Program of the Molecular Biology and Meth[illegible] Development Section, efforts have been concentrated on the development techniques for the analysis of drug effects on proliferative cell cycle progression and for the application of such techniques to the characterization of cancer chemotherapeutic drugs and to the identification of new [illegible] with potential therapeutic activity. The methods employed in this project are mammalian cell [illegible] techniques applied to the growth of tumor in suspension culture; flow microfluorometric determination of cell DNA content of fluorescent-stained cells; and processing, analysis and plotting of cell DNA content distributions by computer.

During the past year, a generalized method has been developed for analysis of wide variety of DNA content distribution profiles obtained by flow microfluorometric determination of DNA content of drug-treated cells. The shapes of these curves were characterized by five parameters and analyzed by two-group discriminant analysis. It has been possible to classify all agents studied as belonging to one of five visually distinguishable curve shapes. This method may be applicable to rapidly characterizing the cell cycle progression properties of large numbers of agents. A continuation of this project is under way to extend it to the evaluation of other therapeutically active agents and to active natural product extracts.

A second major intramural project of this section is exploiting changes in cell surface which accompany neoplastic transformation, with the objective of finding cell division inhibitors which bind selectively to transformed cells. During the current year, efforts have been concentrated on the antitumor antibiotic macromomycin. The crude preparations of this agent originally available were found to show activity against the B_{16} melanoma and the Lewis lung carcinoma, with the site of action being the neoplastic cell membrane. A more highly purified [illegible] antibiotic presently under study has been found to be more active against Lewis lung tumors, but less active against B_{16} melanoma. [illegible] toxicity, therapeutic activity and immunological effects of the purified antibiotic are still in progress.

The extramural contract program of the Molecular Biology and Methods Development Program is described more fully later in this report. In summary, however a major objective of the contract program is to provide biochemical characterization of antitumor drugs under development by the Program as a whole. To this end, contract-supported studies are carried out on the effects of new agents on polynucleotide metabolism, on protein synthesis, on de novo purine and pyrimidine synthesis, on membrane structure and properties, and on electron transport processes involved in aerobic energy metabolism. Additional contract-supported projects are in progress to assess the activity of new agents against tumors of viral etiology, to study the surface effects of sugar analogs exhibiting antitumor activity, and to provide biochemical rationales for the combined use of radiotherapy and clinically established antitumor drugs. Finally, two contract-supported research projects have the objective of providing information on effects of new antitumor agents on cell growth kinetics, by studying the survival of normal hematopoietic stem cells and of malignant cells during drug treatment; and by determining the cell cycle phase-specific progression and cytocidal action of drugs presently

in development for clinical trial.

Apart from its value to the Drug Research and Development Program in its efforts to develop new antitumor agents, the information generated in these contract-supported studies is also utilized in the preparation of IND applications for pre-clinical and clinical trials of new agents, and in the preparation of Molecular Biology Data Sheets which are made available both to basic scientists with an interest in antineoplastic agents, and to investigators involved in the introduction of new antitumor agents into the clinic.

The preceding report summarizes the objectives of the new Laboratory of Medicinal Chemistry and Biology, and outlines some of the research carried out within the Laboratory during its initial year. The individual project reports which follow describe this research in greater detail, and list the publications which have resulted from these studies.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07102-01 LMCB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pharmacology of Maytansine, Dimethylbenzylrifampicin, and other Ansa Macrolides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	M. K. Wolpert	Senior Staff Fellow	DEB, NCI
	F. Mandelbaum-Shavit	Visiting Scientist	LMCB, NCI
	V. H. Bono, Jr.	Head, Molecular Biol. & Methods Dev. Sec.	LMCB, NCI

COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacology, ETB (Project No. Z01-CM-06125-04 LCP)
Laboratory of Experimental Chemotherapy, DR&DB
Medicine Branch, CO, DCT
Laboratory of Pathology, DCBD

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to study the mode of action and the pharmacology of antitumor agents of the ansa macrolide class. Attention is presently being concentrated on the plant product maytansine and its naturally occurring and semi-synthetic derivatives. Maytansine was found to impair microtubule formation in mammalian cells; and to produce metaphase arrest similar to that seen with the Vinca alkaloids. Topics of present interest are a) the nature of the maytansine binding site; b) electron microscopic studies of the alterations in microtubular structure produced by maytansine; c) bioassay methods for maytansine.

PHS-6040
(12-75)

Studies have continued at the in vivo, cellular and subcellular levels on the mechanism of the antitumor action of maytansine, an ansa macrolide isolated from Maytenus serrata by Kupchan and co-workers. Maytansine was cytotoxic to L1210, L5178Y and P388 cell lines in tissue culture in the 10^{-9} and 10^{-8} M concentration range. In contrast to the rifamycins, another series of ansa macrolides, maytansine was an extremely weak inhibitor of E. coli RNA polymerase. As its major cytotoxic effect, maytansine produced metaphase arrest in a manner similar to the Vinca and colchicine alkaloids. The effects of maytansine on DNA, RNA and protein synthesis were also examined, with the most sensitive process being DNA synthesis. However, the effect on DNA synthesis was determined to be secondary, as the rate of incorporation of ^{3}H-thymidine into DNA decreased with time in parallel to the rate of accumulation of cells in mitosis.

In in vivo studies it was found that maytansine was active against the P388 murine leukemia in doses as low as 25 µg/kg ip on a daily x 9 schedule, but was not active against a subline resistant to vincristine, a clinically active anticancer agent. Of particular interest was the finding that maytansine was more potent both in vivo and in vitro than vincristine. In a collaborative study with Dr. R. K. Johnson of the Laboratory of Experimental Chemotherapy, the antitumor properties of maytansine and its ability to cause mitotic arrest in bone marrow, GI tract and ascites tumor cells in mice bearing the P388 tumor were examined. The purpose of these studies was to understand how maytansine affects both normal and tumor tissue, to aid in rational dose scheduling and in the design of combination chemotherapy regimens.

In a collaborative study with Dr. V. H. Bono, the effect of maytansine with time on the mitotic index and DNA content of cultured L1210 cells was studied. It was found that after a 12 hr exposure to maytansine, the mitotic index reached 66.8% and the relative DNA content by flow micro-fluorometry analysis (FMF analysis) shifted to double that found in G_1 cells. Furthermore, 36 hr after treatment, a cell population with four times the G_1 complement of DNA was observed; the discovery of these tetraploid cells, which was confirmed by histologic examination, indicated that many cells had endoreduplicated but had failed to divide into two daughter cells. Since the data suggested that a major cytotoxic effect of maytansine was to disrupt the function of microtubules, studies were designed to determine 1) whether evidence for disruption could be ascertained by electron microscopy and 2) whether evidence for binding of maytansine to tubulin, the major component of microtubules, could be demonstrated.

In collaboration with Dr. W. G. Banfield of the Laboratory of Pathology in the Division of Cancer Biology and Diagnosis of the NCI, it was found by electron microscope (EM) studies that, in L1210 cells grown in suspension culture and treated with maytansine, there was virtually a complete loss of all microtubules. In untreated mitotic cells, microtubules were well formed and well delineated. It was also found by EM analysis that the

antimitotic effects of maytansine were similar to those seen in parallel cultures treated with colcemid, vincristine sulfate and griseofulvin, although maytansine was more potent. Because maytansine produced metaphase arrest, a study was initiated in collaboration with Dr. F. Mandelbaum-Shavit, a Visiting Scientist, to learn how maytansine interacts with microtubules. In a standard assay the binding of ^{3}H-vincristine to rat brain microtubules was measured in a reaction mixture consisting of 0.01 M sodium phosphate buffer, pH 6.5, 0.1 M glutamate, 0.8 mg brain supernatant protein, by absorption of the vincristine:tubulin complex on discs of DEAE-impregnated filter paper. Vincristine binding was temperature-dependent (optimum at 35-37^{o}), pH-dependent (optimum at 6.0-6.5) and exhibited a binding constant (K_A) of 0.5 x 10^{6} M^{-1}. It was found that maytansine competitively inhibited vincristine binding, with a K_i-value of 0.13 µM, suggesting that a quantitative assay for maytansine could be based on the competition between maytansine and ^{3}H-vincristine for binding. Future studies are planned to delineate more clearly the mode of binding of maytansine to purified brain tubulin.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07103-01 LMCB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the Clinical Pharmacology of Folic Acid Antagonists

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

SUMMARY OF WORK (200 words or less - underline keywords)

While methotrexate has been in clinical use for a number of years as an antitumor agent, the recent introduction of high dose schedules followed by citrovorum factor rescue in the treatment of solid tumors has resulted in renewed interest in this agent, and has revealed that our knowledge of the clinical pharmacology of this and related folate antagonists is still incomplete. Topics of present interest are: 1) the role of the metabolite 7-hydroxymethotrexate in the renal toxicity frequently observed with high dose methotrexate; 2) metabolic handling of the folate antagonist aminopterin when administered at high dose levels.

FM-C040

In studies on the pharmacokinetics of high dose methotrexate in patients receiving between 50 and 200 mg/kg, the peak plasma levels were proportional to dose and some 20 to 200-fold higher than that achieved with conventional doses. Peak urine levels were 10-fold greater than the peak plasma MTX levels and exceeded the maximum concentration of MTX soluble in urine at pH 6 or below. Animal studies were designed to test the hypothesis that limited solubility of MTX was responsible for the renal toxicity associated with the high-dose regimen. Histological sections and autoradiographs of tissue slices from the kidneys of monkeys which had received high-dose MTX, and developed the syndrome of renal failure, showed crystalline-like deposits of MTX or MTX-derived material within the lumen of the renal tubules. Chromatographic studies of the urine from human subjects and rhesus monkeys receiving high-dose MTX revealed the presence of a metabolite of MTX, 7-hydroxymethotrexate, which was not seen at lower dose levels. Although 7-hydroxymethotrexate is some two orders of magnitude less effective as an inhibitor of dihydrofolate reducatse than MTX and does not contribute significantly to the folate antagonist activity of MTX, it may be of clinical and toxicological importance because it is some 3 to 5-fold less soluble than the parent compound.

In previous studies of the 7-hydroxylation of MTX by the rabbit, in which species the reaction is quantitatively significant even at lower dose levels, the hydroxylation has been shown to be catalyzed by hepatic aldehyde oxidase. For the purpose of detecting slow 7-hydroxylation of MTX, a radioisotopic assay was developed in this laboratory based on the measurement of tritiated water released when 3',5'-7,9(n)^{3}H-MTX is hydroxylated to yield 3',5'-9(n)^{3}H-7OH-MTX. Using this method, MTX oxidase activity is seen in liver preparations from man, Rhesus monkey and rabbits but not in human kidney.

The diglutamate metabolite of methotrexate, MTX(G_1), was identified in necropsy liver samples from patients who had received MTX. The MTX(G_1) to MTX ratio increased with the length of the interval after the last dose of MTX, suggesting that MTX(G_1) was preferentially retained. By means of competitive binding studies, MTX(G_1) was found to be a more potent inhibitor of human dihydrofolate reductase than the parent compound.

In view of the still increasing interest in the clinical application of methotrexate and related folate antagonists, studies on their clinical pharmacology will be continued. Particular emphasis will be placed on assessing the biological role of the two recently discovered metabolites in man, 7-hydroxymethotrexate and MTX(G_1); and on the metabolic handling of the folate antagonist aminopterin.

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Jacobs, S.A., Stoller, R.G., Chabner, B.A., Johns, D.G.: 7-Hydroxy-methotrexate as a urinary metabolite in human subjects and Rhesus monkeys receiving high dose methotrexate. J. Clin. Invest. 57: 534-538, 1976.

Jacobs, S.A., Stoller, R.G., Chabner, B.A., Johns, D.G.: Dose-dependent metabolism of methotrexate in man and Rhesus monkey. Cancer Treatment Rep. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07106-01 LMCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Adenosine Deaminase Inhibitors as Potentiators of the Pharmacological Activity of Adenosine Analogs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. G. Johns Acting Chief LMCB, NCI

COOPERATING UNITS (if any)
Laboratory of Chemical Pharmacology, ETP, DCT

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

A number of presently available adenosine analogs exhibit antitumor, antiviral and antibacterial activity in vitro, but have proven to be of only limited usefulness in vivo because of their susceptibility to inactivation by the enzyme adenosine deaminase. The purpose of the present study is to determine the effectiveness of adenosine deaminase inhibitors in potentiating the pharmacological effectiveness of these analogs.

FMS-6040

Initial studies have been carried out with the adenosine analog cordycepin (3'-deoxyadenosine), a nucleoside antibiotic which is rapidly converted *in vivo* to the corresponding mono, di- and triphosphate forms. Cordycepin exhibits growth inhibitory properties in bacterial and mammalian test systems and is, in addition, an effective inhibitor of viral replication. The adenosine deaminase inhibitor used in these studies was 2'-deoxycoformycin, a potent inhibitor of this enzyme recently isolated and characterized by Woo and his co-workers (J. Heterocyclic Chem. 11, 641, 1974). 2'-Deoxycoformycin was found to be a highly effective inhibitor of the enzymic conversion of cordycepin to 3'-deoxyinosine, with I_{50}-values of 4.5×10^{-10} M and 6.9×10^{-10} M for adenosine deaminase from L1210 cells and from calf intestine respectively.

To determine the effects of cordycepin, 2'-deoxycoformycin, and combinations of these two agent in mammalian cell culture systems, the compounds were evaluated for growth inhibitory effects against the murine leukemia cell lines L1210 and P388 and the human lymphoblastic leukemia cell line CEM. When added together, cordycepin and 2'-deoxycoformycin resulted in marked inhibition of cell growth at the lowest doses studied (cordycepin 0.1 µg/ml and 2'-deoxycoformycin 1 µg/ml. When cordycepin was added alone, a drug concentration three to four order of magnitude higher (10 - 100 µg/ml) was required to produce a comparable effect. 2'-Deoxycoformycin when used alone at levels of 1 µg/ml and 10 µg/ml was without growth inhibitory effect.

For the assessment of antitumor activity *in vivo*, cordycepin, 2'-deoxycoformycin, and combinations of the two agents were evaluated in CDF$_1$ male mice bearing the P388 leukemia. Cordycepin alone was only marginally active in this test system, but resulted in significant prolongation of survival when administered in combination with 2'-deoxycoformycin. 2'-Deoxycoformycin alone was without antitumor activity.

Studies are presently being carried out to determine whether 2'-deoxycoformycin exerts a similar synergistic effect when administered with other adenosine analogs known to be subject to inactivation by adenosine deaminase.

Publications:

Johns, D.G. and Adamson, R.H.: Enhancement of the biological activity of cordycepin (3'-deoxyadenosine) by the adenosine deaminase inhibitor 2'-deoxycoformycin. Biochemical Pharmacology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 07105-01 LMCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Biochemical and Pharmacological Studies of Pyrimidine Nucleoside Analogs with Antitumor Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Voytek	Senior Staff Fellow	LMCB, NCI
OTHER:	D. G. Johns	Acting Chief	LMCB, NCI
	J. S. Driscoll	Head, Drug Design and Chemistry Section	LMCB, NCI
	M.K. Wolpert-DeFilippes	Senior Staff Fellow	DEB, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long-range aim of this project to study the pharmacological activity and biochemical mode of action of new antitumor agents of the pyrimidine nucleoside class. The topic of present interest is the compound 5,6-dihydro-5-azacytidine, a reduced derivative of 5-azacytidine with greater chemical stability than the latter compound.

The nucleoside 5-azacytidine shows significant antitumor activity in patients with acute myelocytic leukemia. However, the therapeutic use of this agent is hampered by its chemical instability in aqueous solution. In addition, 5-azacytidine is rapidly inactivated *in vivo* by the enzyme cytidine deaminase. A number of analogs of 5-azacytidine have been synthesized in this Laboratory in an attempt to obtain compounds which retain the antitumor activity of 5-azacytidine without the disadvantages of the latter. The most promising of these derivatives, 5,6-dihydro-5-azacytidine, possesses antitumor activity in the murine L1210 leukemia system, and shows greater stability in aqueous solution than does the parent compound. Like 5-azacytidine, 5,6-dihydro-5-azacytidine is deaminated by cytidine deaminase, but at a slower rate; the deamination is inhibited by tetrahydrouridine.

Studies are in progress to determine whether the biochemical mode of action of 5,6-dihydro-5-azacytidine is identical to that of 5-azacytidine, and whether it displays any significant therapeutic advantage over the latter in experimental tumor systems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07107-01 LMCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Biological Studies on Metastatic and Non-Metastatic Melanomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. R. Breitman	Research Chemist	LMCB, NCI
OTHER:	G. H. Sato	Prof., Biology Dept.	Univ. of Calif.
	J. Mather	Post-doct. Fellow, Biology Dept.	Univ. of Calif.
	P. Roberts	Research Assistant, Biology Dept.	Univ. of Calif.

COOPERATING UNITS (if any)
Department of Biology, University of California, San Diego, California

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to find differences between metastatic and non-metastatic tumors which can be exploited with chemotherapeutic of other methods. The system under study is the transplantable B_{16} mouse melanoma. Cell lines have been isolated from this tumor having various metastatic properties. The growth requirements of these cell lines in tissue culture are being investigated with the anticipation that difference in metastatic behavior will be correlated with differences in growth requirements.

PHS-6040
(12-75)

Objectives:

To establish in tissue culture cell lines from the B_{16} mouse melanoma which have various metastatic characteristics and to define the growth requirements of these lines.

Major Findings:

Clonal lines of the B_{16} tumor have been isolated which differ in their degree of metastatic behavior. These cell lines have been designated as S6 (highly metastatic with a metastatic rate of 80-100%), clone 2 (a metastatic rate of 50%), clone 10 (a metastatic rate of less than 3%), and clone S6 bc (isolated from the blood of animals subcutaneously injected with S6 but which cannot metastasize when injected either s.c. or i.v.). All these cell lines are pigmented both in culture and as tumors. These cell lines are now a catalog to be compared one to the other.

One question of interest to us has been the host range of the highly metastatic S6 melanoma cell line and whether this line metastasizes in athymic nude mice. It was found that a s.c. injection of 10^6 S6 cells gave rise to tumors in Balb/c and the athymic strains of Swiss and Balb/c. However, there were no metastatases. In contrast in C57 Bl/6 and athymic C57 Bl/6 mice had tumors with metastatases. These results eliminate T cell lymphocytes as having an involvement in this metastatic process. We are now trying to establish a breeding colony of athymic nude C57 mice to continue these experiments.

Mose of the efforts are now involved in defining the growth requirements in tissue culture of melanoma cells. At this time we are concentrating on S6 (highly metastatic) and Clone 10 (non-metastatic). Optimal growth of both cell lines requires the addition of serum to the basic nutrient medium. In the absence of serum the cells die rapidly. We have succeeded in obtaining approximately 20% of the growth obtained in serum-medium by the addition of various hormones and growth factors to serum-free medium. Clone 10 requires insulin, transferin, and FSH. The requirements of S6 include the above plus progesterone or testosterone and LRF. About twenty other hormones and growth factors have been tested with negative results. An interesting observation is that S6 has a much greater reducing requirement, i.e., replacement of cysteine with cystine results in a marked inhibition of growth in serum-free medium. This effect can be partially reversed by the addition of B-mercaptoethanol.

Significance to Biomedical Research and Proposed Course;

This project has as an underlying principle that at this time it may be of greater utility to understand and therefore be able to treat metastasis than to understand the nature of tumorigenesis. Certainly, a very large percent of the efforts in cancer research is involved with the question of what are exploitable differences between normal and tumor cells. My

approach has been to find exploitable differences between metastatic and non-metastatic tumors. The establishment of melanoma cell lines with different metastatic properties provides an excellent experimental system to determine the different growth requirements of these cells. Tissue culture is a very powerful tool for this undertaking. We have been very excited that at essentially the initial stages of this work differences between highly metastatic and non-metastatic cell lines have been seen. Efforts have now begun to identify those factors in serum that are required for the growth of the melanoma cell lines.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07104-01 LMCB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Nutritional and Pharmacological Regimens for Potentiation of Chemotherapeutic Activities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Rabinovitz	Research Chemist	LMCB, NCI
OTHER:	F. J. Schiffman	Research Associate	LMCB, NCI
	D. T. Vistica	Pharmacology Research Associate	LMCB, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to study the feasibility of nutritional and pharmacological intervention to promote the activity of established and potential cancer chemotherapeutic agents, and to increase their selectivity for tumor cells. Approaches to this goal include the following: 1) alteration of the nutritional status of tumor cells, both in culture or in vivo, to produce conditions interfering with the metabolic inactivation of the chemotherapeutic agent. This approach will be particularly directed against resistant cell lines; 2) modification of the cell membrane of susceptible and resistant varieties of tumor cells by the use of either available or newly synthesized membrane-reactive agents; 3) synthesis of analogs of known agents in order to introduce modifications to make them more suitable for the regimens described above.

Major Findings:

The selectivity of the fungicidal polyene antibiotic, amphotericin-B, is considered to be due to the presence of ergosterol in the membranes of susceptible fungi. We have investigated the effect of ergosterol added to the growth medium of L1210 cells on the subsequent sensitivity of these cells to amphotericin-B. Cells grown in ergosterol showed increased sensitivity to the lytic action of the antibiotic. Cholesterol at equivalent levels was ineffective and at a concentration of amphotericin-B which was not cytotoxic to cells grown in cholesterol, over a two log kill was observed with cells grown in ergosterol.

The lethal action of melphalan on L1210 cells *in vitro* was shown to be determined by the amino acid content of the medium. Various amino acids protected the cells from the onset of cytotoxicity to different extents, and this protection was both concentration and time dependent. Present evidence suggests that the protection is mediated through competition with melphalan for a high affinity transport site.

The amino acids affording protection do not correspond to those reported in the literature to be transported by a high affinity mechanism in other cell types. This suggests that the establishment of both an *amino acid protection profile* for tumor cells *in vitro* and another profile for protection against acute toxicity in the host, may permit the formulation of an amino acid mixture which when injected with melphalan into the tumor bearing animal would both protect the host and direct the agent to the tumor.

Significance for Cancer Research and the Program of the Institute:

These studies fall under Objective 6, Approaches 1 and 2 of the National Cancer Plan, --"to develop and utilize methodologies of chemotherapy in the cure and palliation of cancer and to discover new therapeutic agents and develop means for their selection".

Drug Design and Chemistry Section

Laboratory of Medicinal Chemistry and Biology

July 1, 1975 - June 30, 1976

Scope

The objectives of the Drug Design and Chemistry Section of the Laboratory of Medicinal Chemistry and Biology are: (1) chemical research in the design and synthesis of new drugs for the treatment of cancer, (2) the chemistry of agents and drugs of interest in the treatment of cancer, (3) research on the structure, purity, decomposition products and metabolites of antitumor drugs by analytical instrumental techniques, and (4) the development of structure-activity techniques to summarize existing data and to provide guidelines for analog synthesis.

Staff

The staff consisted of three scientists, one Senior Staff Fellow, five NIH Visiting Fellows and a secretary. In addition, several persons are employed on the College Work-Study Program as part-time technicians.

Summary of Accomplishments

Approximately five man-years effort was devoted to the intramural chemical research projects described in Individual Project Reports ZO1-CM-03580, ZO1-CM-03581, and ZO1-CM-03582. An area of primary medicinal chemical interest is central nervous system antitumor agents. Spirohydantoin mustard (NSC-172112) was made DN2A based on its murine brain tumor activity. The pharmacology of this compound is presently being studied by the NCI. A second type of agent, an aziridinylbenzoquinone which was designed and synthesized for its CNS antitumor properties, has been selected for presentation as a potential DN candidate. This compound is active in the following tumor models (%ILS): B16 melanoma (70%), P388 (138%), IPL1210 (169%), ICL1210 (80%), and IC ependymoblastoma (>348%; 4 of 6 90 day survivors).

A significant discovery in another anticancer agent area involved the synthesis of a 5-azacytidine analog which remains L1210 active (ILS > 100%) but is very stable in aqueous solution. This compound may be useful under the infusion conditions employed with the hydrolytically unstable parent drug. Complexes between protein and antitumor drugs were prepared using a number of clinically useful drugs. In several instances,improved murine antitumor activity and/or therapeutic indices were observed relative to the uncomplexed drug in the same experiment.

A high resolution mass spectrometer utilizing a gas chromatographic inlet was made operational. It has proven exceptionally useful in organic structure elucidation studies and is also being utilized in the structural determination of antitumor drug metabolites.

Section personnel were also involved in the extramural contract research program. They served as project officers on a Pomona College contract which has the objective of correlating chemical structure with antitumor activity in a quantitative manner. The adriamycin, mercaptopurine, terephthanilide and fluorouracil families are among those currently under investigatrion.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03580-07 LMCB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Chemical Research in the Development of New Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. S. Driscoll	Head, Drug Design and Chem. Section	LMCB	NCI
OTHER:	J. A. Beisler	Research Chemist	LMCB	NCI
	M. M. Abbasi	Visiting Fellow	LMCB	NCI
	F. E. Chou	Visiting Fellow	LMCB	NCI
	D. R. Hwang	Visiting Fellow	LMCB	NCI
	K. Okada	Visiting Fellow	LMCB	NCI
	G. R. Proctor	NCI Visitng Expert	LMCB	NCI

COOPERATING UNITS (if any)

Drug Evaluation Branch, DR&DP, DCT, NCI
Lab. Chem. Pharmacology, EXT, DCT, NCI
L. Eotvos Univ., Budapest, Hungary

LAB/BRANCH

Laboratory of Medicinal Chemistry & Biology

SECTION

Drug Design and Chemistry

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.1	4.1	

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is the discovery of new types of clinically useful anticancer drugs. The following topics are of current interest: (1) reduced nucleosides including stable analogs of 5-azacytidine, (2) potential central nervous system antitumor agents, e.g. aziridinylquinones, hydantoin derivatives, aliphatic procarbazine analogs, (3) potential lung tumor agents, (4) protein-antitumor drug complexes, (5) "masked" agents capable of generating small reactive molecules in vivo and (6) pyridones and quinoneimines.

Project Description:

General Objective:

The objective of this project is the discovery of new types of clinically useful anticancer drugs. Medicinal chemical research is directed towards the synthesis of new compounds which have potential as useful agents. Leads for this program are generated from structure-activity studies, the DR&DP screening program and the literature.

Specific Objectives:

1. synthesis of a chemically stable form of 5-azacytidine which will be suitable for clinical infusion studies
2. design and synthesis of new agents having activity against tumors of the central nervous system
3. development of new agents having activity against lung tumors
4. improvement of the properties of known antitumor drugs through complexation with proteins
5. synthesis of "masked" agents capable of in vivo decomposition to cytotoxic compounds; agents based on screening leads

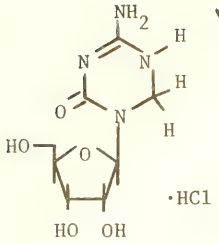
Major Findings:

A. Dihydro-5-azacytidine (Drs. Beisler, Abbasi, Driscoll)

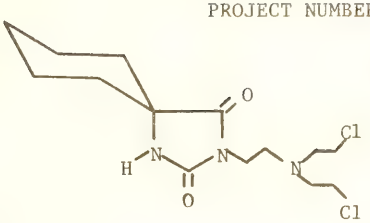
5-Azacytidine (NSC 102816) is a clinically useful agent which is usually administered by infusion to minimize gastrointestinal toxicity problems. Unfortunately, this material is unstable in aqueous solutions, giving a variety of decomposition products possessing either different or unknown activity and toxicity characteristics. Since the decomposition reaction is known from literature data to take place by nucleophilic (H_2O) attack at the 6-position of the triazine ring, several approaches were devised with the objective of protecting the molecule against that reaction. Reduction of the 5,6-double bond was one of these planned approaches.

5,6-Dihydro-5-azacytidine hydrochloride (A-1) was prepared by chemical reduction of 5-azacytidine. This compound was completely characterized both as the hydrochloride salt (NSC 264880) and the free base (NSC 265483) by gas chromatography, mass spectrometry, N.M.R., U.V. and I.R. spectroscopy and elemental analysis. Acid hydrolysis of (A-1) removed the sugar and produced 5,6-dihydro-5-azacytosine hydrochloride. This compound (NSC 265485) was identical with that produced by the reduction of 5-azacytosine.

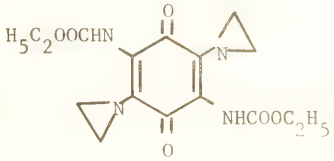
While solutions of 5-azacytidine in water undergo 25-40% decomposition after 24 hours at room temperature, dihydro-5-azacytidine hydrochloride (A-1) appears to undergo no decomposition under similar conditions over a period of 60 days.



A-1



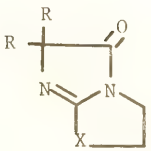
B-1



B-2

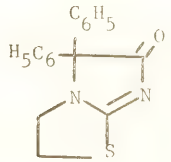
$CH_3NHNH(CH_2)_nCONHCH(CH_3)_2$

B-3

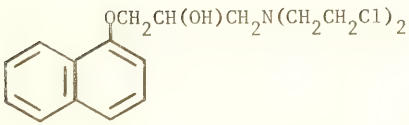


B-4

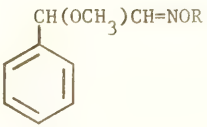
X=O,S



B-5

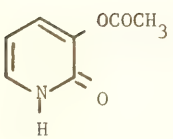


C-1



E-1

E-2



E-3

E-4

Limited antitumor activity data are available at the present time. Two separate in vivo L1210 tests (Q4D schedule) have been completed. In both experiments, maximum activity was obtained at the top dose tested (400 mg/kg, ILS 62% and 600 mg/kg, ILS 104%). U.V. analysis shows less than 0.06% of 5-azacytidine in our samples of dihydro-5-azacytidine. 5-Azacytydine run in parallel experiments gave ILS values of 144 and 138% at 10 and 20 mg/kg respectively. Compound A-1 had a therapeutic index (highest active dose divided by lowest active dose) of 50 in these Q4D experiments and has a water solubility greater than 200 mg/ml.

The optimum treatment schedule is presently being established for A-1. In vivo tests are then planned in the B16 melanoma, P388 leukemia and the 5-azacytidine resistant P388 tumor systems as well as other appropriate tumor models. Tests are currently underway in both cell culture and enzymatic systems. Additional synthetic work is in progress in the area of reduced nucleosides including tetrahydro-5-azacytidine and reduced Ara-C. It is also hoped that several new chemical reactions discovered during the course of the 5-azacytidine work will allow the synthesis of arabinosyl-5-azacytosine which the literature indicates is not presently attainable.

B. Potential CNS Antitumor Agents

1. Spirohydantoin Mustard (Drs. Driscoll and Okada): This compound (B-1, NSC 172112) was classified 2A in the decision network process during the past year on the basis of its murine intracerebral ependymoblastoma and intraperitoneal P388 activity. The synthetic method was improved to produce B-1 free from a previously encountered dimeric impurity and a purification method was devised to remove the dimer from previously prepared samples. A pure sample of an intermediate in the synthetic sequence was synthesized and supplied to a contractor for the preparation of radiolabeled material. A water soluble hydrochloride salt was prepared. While the hydrochloride is still under study, other synthetic work is not planned at the present time.

2. Diaziridinylquinones (Drs. Chou and Driscoll): Synthetic work and antitumor testing on the diaminodiaziridinylbenzoquinone family was completed. The objectives were the preparation of compounds possessing both murine CNS activity and adequate water solubility. Nineteen carefully selected compounds were synthetized. Eight of these compounds gave 60 day survivors in the intracerebral (IC) ependymoblastoma tumor model in more than one experiment. ILS values in excess of 200% were observed in each of these experiments. Five compounds reproducibly gave intraperitoneal (IP) L1210 activities of ILS > 100% with long term (30 day) survivors. Five showed substantial (ILS reproducibly > 50%) IC L1210 activity. As would be expected from the L1210 results, 30 day survivors were seen in the IP P388 test system. Four members of this family were reproducibly active in the B16 melanoma system. Optimum doses ranged from 0.1 to 25 mg/kg while water

solubilities ranged between 60 and < 0.1 mg/ml. One of the overall most active compounds was the urethane, B-2 (NSC 182986) which had a water solubility of ca. 0.5 mg/ml and the following activities (tumor; ILS): IP L1210, 169%; IC L1210, 84%; IP P388, 138%; IC P388, 51%; IP B16, 70%; IC ependymoblastoma, 348% (four of six 99 day survivors).

Redox potentials and partition coefficients are being measured in this series in order to attempt a Hansch-type quantitative structure-activity analysis. Further synthetic work is not planned unless the structure-activity study indicates that a significant potential gain is possible.

3. Aliphatic Procarbazine Analogs (Dr. Beisler): The synthesis of three derivatives with n = 2,3 and 4 (B-3) has been completed and the compounds submitted for testing. The compound (B-3, n = 4) would be expected to have approximately the same partition coefficient as procarbazine. Additional synthetic work will be carried out only if these analogs show antitumor activity.

4. Bicyclic Hydantoins and Levamisole Analogs (Dr. Okada): Because of its CNS penetration ability and a general structural similarity to levamisole, diphenylhydantoin was used as a framework for the preparation of selected analogs with antitumor potential Three compounds of the type B-4 were synthesized. Although possessing alkylating properties, the oxygen analogs were L210 inactive. In vivo results are not yet available on the sulfur analog (B-4, R = C_6H_5, X = S). These compounds are also being tested as possible alkaline phosphatase inhibitors at Yale Medical School. The production of a levamisole analog by carbonyl reduction is in progress. Also prepared was B-5 with the isolevamisole skeleton. Work in this area will be terminated unless the biological test results are encouraging.

C. Potential Lung Antitumor Agents

1. Lung specific agents for antitumor evaluation (Dr. Beisler and Ms. Abbott): Approximately 25 drugs with clinical activity against pulmonary tuberculosis are being tested against the I.V. Lewis lung tumor system. If any of these drug classes show antitumor activity, the lead will be pursued further through acquisitions from drug companies and an intramural synthesis program.

2. Propranolol mustard (Drs. Beisler and Adamson): From among the numerous drugs which concentrate in the lungs, propranolol was chosen for modification with an alkylating groups (C-1) in the hope that the resulting compound would be antitumor active and still tissue specific. Since only one optical isomer of propranolol exerts β-blockage, a nitrogen mustard derivative of the other isomer might serve as an antitumor agent without propranolol activity as a side effect. Two steps of the 3 step synthetic sequence leading to racemic C-1 have been accomplished.

D. Protein-antitumor drug complexes (Drs. Szekerke and Driscoll)

Forty antitumor drug-protein complexes utilizing various combinations of 5-azacytidine, ara-C, actinomycin D, an active acridine derivative, cytembena, dichloro-MTX, ellipticine and AT-125 with albumin, fibrinogen and chemically modified albumin were evaluated in the L1210 system (cytembena complexes-P388). Some covalent as well as ionic or charge-transfer complexes were prepared. The parent compound was always evaluated in the same experiment with the complex. Increases in ILS values with the complexes relative to the uncomplexed drug were observed for numerous combinations. Also observed was (1) an increase in solution stability (5-azacytidine) and (2) an apparent increase in water solubility (acridine NSC 141549). Cooperative studies (L. Eotvos Univ., Budapest and NCI) are continuing in this area.

E. "Masked" Agents and Development of Screening Leads

1. Compounds capable of producing small reactive molecules in vivo (Dr. Proctor): (a) HCN generators - Oxygen substituted benzaldoximes were prepared as potential HCN generators. Additional substituents (e.g. methoxy, E-1) were chosen to maximize the fragmentation pathway producing HCN at the expense of the organic nitrile pathway. HCN has been produced chemically from E-1 derivatives. Also generated were the corresponding nitrile and benzaldehyde. The quantitative aspects of the reaction are being studied by gas chromatography (gc) and gc/mass spectrometry. A number of derivatives have been submitted for in vivo antitumor testing. Additional synthetic work is planned only if these compounds are active. (b) Ethylene oxide generators (Dr. Proctor) - N-Tosyl-N-nitroso-2-chloroethylamine was prepared as an intermediate in the preparation of the presently unknown Δ^2-1,2,3-oxadiazoline ring system (E-2). This system, which is a theoretically possible decomposition product from the 2-chloroethylnitrosoureas, might generate the alkylating agent, ethylene oxide, and nitrogen upon in vivo decomposition. Several relatively simple, but highly unstable, ring systems are currently under study as in vivo generators of cytotoxic agents.

2. Pyridones (Drs. Hwang and Proctor): Four pyridones from the random screen have shown moderate, reproducible L1210 and P388 activity. The synthesis of a series of analogs of one of these compounds, 3-acetoxy-2-pyridone (E-3), has been begun in order to explore the structure-activity characteristics of the series and maximize antitumor activity. General synthetic procedures have been developed to O-acylate the oxygen atom in the 3-position of 2,3-dihydroxypyridine and to N-alkylate this compound.

3. Quinoneimines and their hydroxylamino and hydrazine analogs (Dr. Chou): A random screening lead developed a number of years ago indicated that E-4 ($R = NH_2$) had moderate but reproducible L1210 activity. Because of our interest in both quinone and hydrazine analogs, a program was initiated to develop a synthetic method for this unpublished compound and to explore the structure-activity relationships in this series. During the course of the

work, it was discovered by another group that the structure of the original "lead" compound had been incorrectly assigned. At this point, a number of hydroxylamine analogs (E-4, R = OH, OTs etc.) had been prepared and submitted for testing. It was subsequently found that none of these materials possessed P388 activity. This project has been terminated.

Publications

Peng, G.W., Marquez, V.E. and Driscoll, J.S.: Potential central nervous system antitumor agents. Hydantoin derivatives. J. Med. Chem. 18: 846-849, 1975.

Khan, A.H. and Driscoll, J.S.: Potential central nervous system antitumor agents. Aziridinylbenzoquinones 1. J. Med. Chem. 19: 313-317, 1976.

Hirata, T. and Driscoll, J.S.: Potential central nervous system antitumor agents. Phenothiazines I. Nitrogen mustard derivatives. J. Pharm. Sci., (in press).

Driscoll, J.S.: Antineoplastic agents. In Clarke, F.H. (Ed.): Annual Reports in Medicinal Chemistry 1975, (in press).

MITHCONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (DO NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03582-02 LMCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Structure-Activity Studies of Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. R. Quinn	Chemist	LMCB NCI
OTHER:	J. S. Driscoll	Head, Drug Design and Chem. Section	LMCB NCI

COOPERATING UNITS (if any)
Stanford Research Institute
Pomona College
Drug Evaluation Branch, DR&DP, DCT, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Design and Chemistry

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are: (1) the development of quantitative structure-activity correlations among antitumor agent congeners, (2) the development of general structure-activity relationships among large numbers of unrelated antitumor agents and (3) the use of the correlation data obtained to generate leads for new synthesis and increase the rationale for the testing of new agents. Compound families of current interest are adriamycin, terephthalanilide and aziridinylbenzoquinone analogs.

PHS-6040
(12-75)

Project Description:

General Objectives:

The objectives of this project are:

1. the development of quantitative correlations of screening data with structural features among antitumor agent congeners
2. the investigation of structure-activity techniques as applied to antitumor data
3. the development of correlations between structure and antitumor activity among large numbers of structurally unrelated compounds
4. the use of the correlations obtained to: (a) generate leads for new synthesis, (b) increase the rationale for selection of new compounds to be tested and (c) reduce the number of synthetic analogs necessary to obtain the optimally active member of a new series.

Methods Employed:

Computer techniques are used to analyze and organize biological screening data. These data are then correlated with molecular structural parameters using computerized regression analysis techniques.

Major Findings:

A. Quantitative Structure-Activity Relationships by the Hansch Technique (Dr. Quinn)

1. Adriamycin analogs: A cooperative study is underway with Pomona College and Stanford Research Institute to correlate molecular structure with in vivo and in vitro antitumor activity in this family of anthracycline antibiotics.

2. Terephthalanilides (Wander compounds): The antitumor activity of 22 Wander compounds with L1210 ILS values of at least 80% on the chronic treatment schedule was found to be well correlated (r = 0.92) with structural parameters. A molecular length parameter was confirmed as an important variable. The correlation equation also indicates that a certain amount of hydrophilic character is also important.

3. New dependent variables for the Hansch equation (Dr. Quinn): The possibility that the usual dependent variable in the Hansch equation {-log(molar concentration)} might be replaced with one more suitable for cancer data correlations was explored. The use of log ILS was applied to the biological data from a family of Cain acridines. Although a preliminary correlation was obtained in partition coefficient, molar refractivity and sigma values, additional work is required to determine whether log ILS can be developed into a useful dependent variable.

B. Aziridinylbenzoquinones (Drs. Driscoll and Geran)

Reproducible screening data values were obtained for thirty-nine aziridinylbenzoquinones in the intracerebral L1210 and ependymoblastoma systems as well as the intraperitoneal L1210, P388 and B16 tumor models. Several compounds gave cures in at least one tumor system. A candidate for presentation to the Drug Evaluation Committee will be chosen from among these compounds.

Publication

Quinn, F.R., Driscoll, J.S. and Hansch, C.: Structure-activity correlations among Rifamycin B Amides and hydrazides. J. Med. Chem. 18: 332-339, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (DO NOT USE THIS SPACE)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 03581-07 LMC

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Analytical Chemistry of New Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Kelley	Sr. Staff Fellow	LMCB	NCI
OTHER:	J. S. Driscoll	Head, Drug Design and Chem. Section	LMCB	NCI

COOPERATING UNITS (if any)

Laboratory of Toxicology, EXT, DCT, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Design and Chemistry

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is the research and development of analytical techniques which are used to: establish the structure and purity of new antitumor agents and their metabolites, determine physical and chemical properties of new anticancer drugs and study reaction mechanisms. Mass spectrometry, gas-liquid chromatography and the combination of these techniques is emphasized. Compounds of current interest are 5-azacytidine, alanosine, hydantoins, aldoximes and aziridinylquinones.

Project Description:

General Objectives:

The objective of this project is the research and development of analytical techniques which are used to:

1. establish the structure and purity of new anticancer drug candidates;
2. establish structures of metabolites of new antitumor agents;
3. determine important physical and chemical properties of new anticancer drugs; and
4. study reaction mechanisms

Methods Employed:

Mass spectrometry (ms), gas-liquid chromatography (gc) and the combination of these techniques (gc/ms) are emphasized. Other analytical methods such as high pressure liquid chromatography, NMR, UV and IR spectroscopy also are employed.

Major Findings:

A. Dihydro-5-azacytidine (Dr. Kelley)

The reduction product of 5-azacytidine was established as a dihydro derivative by gc/ms analysis. When a deuterated reducing agent was used to produce the reduced nucleoside, the gc/ms technique provided evidence that the reduction occurred at the double bond in the triazine ring. A novel oxidation method using a silation reagent was discovered. This method converts dihydro-5-azacytidine back to the parent compound. This technique may be applicable to the synthesis of deuterium or tritium labeled parent drugs from their partially reduced derivatives.

B. Hydantoins (Dr. Kelley)

1. Spirohydantoin mustard (NSC 172112): Gc/ms analysis was used successfully to identify and quantitate impurities in samples of NSC 172112 prepared by various routes.

2. N-Alkylhydantoins: A gc/ms analytical method was developed to distinguish between the 1- and 3-(mercaptoalkyl)hydantoins produced by hydrolysis of bicyclohydantoin derivaties. This helped establish the structures of the bicyclo compouns.

C. Aldoxime Fragmentation (Dr. Kelley)

A gc analytical system was established to determine the products resulting from the decomposition of a "masked" HCN generator. Hydrogen cyanide as well as other decomposition products were identified and quantified.

D. Alanosine Metabolites (Drs. Kelley and Cooney)

Research is underway to elucidate the structure of a murine urinary metabolite of alanosine by the gc/ms technique.

Publication

Cooney, D.A., Driscoll, J.S., Milman, H.A., Jayaram, H.N. and Davis, R.D.: Inhibitors of L-asparagine synthetase *in vitro*. Cancer Treat. Rep. (in press)

Molecular Biology and Methods Development Section

Laboratory of Medicinal Chemistry and Biology

July 1, 1975 - June 30, 1976

The Molecular Biology and Methods Development Section (MBMD) of the Laboratory of Medicinal Chemistry and Biology is staffed by four senior professionals (including two commissioned officers, and one senior staff fellow) and three technical and clerical employees. The principal objective of this section is to conduct basic and developmental research leading to the establishment of new and improved biological and biochemical drug evaluation assays by coordinating both intra-mural and contract supported laboratory programs. Specifically, this involves basic and developmental research (a) to establish new and improved biochemical, molecular biological, and cell biological drug evaluation assays to characterize therapeutically active drugs of new structure and congeners of drugs of clinical interest to provide the basis for a more advantageous use of these drugs in single drug, combined drug or combined modality therapy, (b) to develop new and improved systems for the primary detection of new drugs by means of in vitro systems including cell culture, and by means of the characterization of the biochemical and cell proliferative kinetic properties of new in vivo tumors systems, and (c) to develop new drugs by means of projects that require concurrent basic research and drug synthesis, and by means of projects that require the isolation, preparation, and characterization of macromolecules with potential therapeutic activity.

Significant Accomplishments (1975-76):

Direct Contract Activities

The section has primary or significant responsibility for 16 research contract projects designed to achieve its objectives. These contracts and their objectives are listed below.

Southern Research Institute (NO1-CM-43784). Objective: This contract has five separate but interrelated objectives: (a) to determine if new antitumor drugs have biochemical activities similar to clinically established drugs, (b) to provide clear leads to biologically significant biochemical properties of new antitumor drugs, (c) to provide details, for selected drugs, of effects on de novo purine and pyrimidine biosynthesis, (d) to develop systems to select structural analogs having more desirable biochemical properties and, (e) to develop biochemical rationales for the combined use of clinically established antitumor drugs.

Seven other contracts have in common, the objective of providing detailed biochemical characterization of antitumor drugs. They differ in the particular area of biochemical expertise. Their common significance to the Division of Cancer Treatment program is to provide the possibility of rational approaches to analog development, therapeutic applications and drug combinations.

University of Texas (NO1-CM-43786). Objective: Details of effects on polynucleotide metabolism.

Yeshiva University (NO1-CM-43787). Objective: Details of effects on protein synthesis.

Brigham Young University (NO1-CM-43790). Objective: Details of effects on folate metabolism.

University of Madrid (NO1-CM-53792). Objective: Effects on electron transport processes in aerobic energy metabolism.

University of Minnesota (NO1-CM-43788). Objective: Mechanisms of drug uptake and effects on membrane transport processes.

Michigan Cancer Foundation (NO1-CM-53789). Objective: Details of effects on membrane structure and properties.

University of Pennsylvania (NO1-CM-53830). Objective: To investigate the bioenergetic aspects of cancer chemotherapeutic agents.

Four of the contract projects are designed to provide special biochemical information of potential use to drug development.

University of California (NO1-CM-43731). Objective: To provide biochemical rationales for the combined use of radiotherapy and clinically established antitumor drugs.

Harvard University (NO1-CM-53825). Objective: To study the cell surface effects of antitumor sugar analogs.

New York State Department of Health (NO1-CM-43785). Objective: To develop an *in vitro* cell growth profile of drug interactions.

Litton Bionetics (NO1-CM-33741). Objective: Screening of compounds for anti-enzymatic, anti-viral, and antitumor activity.

Four contract projects have as their common objective to provide information on the details of effects of drugs on cell biology and growth kinetics. These include both cell culture and *in vivo* systems.

Washington University (NO1-CM-43713). Objective: To study the interaction *in vivo* of anticancer agents at the cellular level by measuring the survival of normal hematopoietic stem cells and of malignant cells.

Energy Research and Development Commission (YO1-CM-10056). Objective: To determine the cell cycle phase specific progression and cytocidal action of clinical drugs and of drugs in development for clinical trials.

Two contract projects, which are not the direct responsibility of this section, have components which support its cell biology objectives. One, Energy

Research and Development Commission (YO1-CM4-0102), in addition to providing the NCI with a Los Alamos flow microfluorometer and cell sorter, is involved in developing methods for solid tumor sample preparation and in studying experimental tumor cell populations for tumor age dependent changes in cell DNA content distributions. The second, University of Texas (NO1-CM-61156), is a pharmacology project. One component of this project is the evaluation of drugs in vitro, on cell cycle phase progression and cell cycle phase cytocidal properties.

Workshops, Symposia, and Reports

Staff members of MBMD were responsible for organizing two workshops, one on Molecular Biology of Drug Action and one on Cell Biology of Drug Action at the annual DCT Contractors Meeting. In addition they were responsible for presentations at the annual Screening Contractors Meeting, and the Sixth and Seventh New Drug Seminars on DTIC and the Nitrosoureas.

Staff members of MBMD has written reports, "Cell Biology and Biochemistry Data Sheets", on 20 drugs of high priority to the DCT drug development and evaluation program for presentation to Decision Point and Clinical working groups.

Intra-mural Research Projects

Two individual projects have been conducted within our intramural laboratories. One project (Project No. ZO1-CM-03579-02-LMCB) titled, "Effects of Chemotherapeutic Agents on L1210 Cell Cycle Progression" is oriented to the development of flow microfluorometric techniques and their application to the in vitro characterization of therapeutic drugs. The second project (Project No. ZO1-CM-3583-04-LMCB), titled, "Modification of Cell Surfaces to Limit Cell Division and Increase Immunogenicity" is aimed at exploiting those changes in cell surface which accompany neoplastic transformation with the objective of finding cell division inhibitors which selectively bind to transformed cells.

Publications: See current report, Laboratory of Medicinal Chemistry and Biology, Office of the Chief.

COMMITTEE ON SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-03579-03 LMCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effects of Chemotherapeutic Agents on L1210 Cell Cycle Progression.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:

Vincent H. Bono, Jr.	Head, Molecular Biology and Methods Development Section	LMCB	NCI
Robert L. Dion	Chemist	LMCB	NCI

Others:

David Abraham	Senior Scientist	LMCB	NCI

COOPERATING UNITS (if any)
Laboratory of Experimental Chemotherapy, NCI
Laboratory of Physical Biology, NIAMD
Laboratory of Molecular Biology, NIAMD

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1	2.5

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the development of techniques for the analysis of drug effects on proliferative cell cycle progression and for their application to the characterization of cancer chemotherapeutic drugs and the identification of agents with potential therapeutic activity. The major methods employed in this project are (a) mammalian cell culture techniques applied to the growth of L1210 leukemia cells in suspension culture, (b) flow microfluorometric determination of cell DNA content of fluorescent stained cells in a Cytofluorograf (Biophysics Systems, Inc.) and (c) processing, analyzing, and plotting cell DNA content frequency distributions by computer.

PHS-6040
(12-75)

Project Description:

During the past year we have continued to screen both therapeutic agents and cytotoxic agents for their effects on cell cycle progression. A method was developed to analyze the DNA content distribution profiles obtained by flow microfluorometric determination of cell DNA content. The shapes of these curves were characterized by five parameters and analyzed by two group discriminant analysis. All agents studied have been classified as belonging to one of five visually distinguishable curve shapes. This method may be applicable to rapidly characterizing the cell cycle progression properties of large numbers of agents. A continuation of this project is planned to extend it to the evaluation of all other therapeutically active agents and to active natural product extracts.

Studies directed toward improving and understanding the basis of various methods for fluorescent staining of cells for DNA content were conducted. The previously used method of fluorescent-Feulgen staining has been discontinued in favor of the propidium iodide staining of cells in hypotonic citrate (Krishan: J. Cell Biol., 66: 188, 1975). We have found a good correlation between the two fluorescent staining techniques and two classical chemical methods for DNA determination (the diphenylamine method of Burton and the method of Ceriotti) in L1210 cells. This was done in collaboration with Dr. Kempner of the Laboratory of Physical Biology, N.I.A.M.D.

A two parameter analysis of cells for DNA content and T-antigen content of SV40 transformed cells is underway in collaboration with Dr. Martin, Laboratory of Molecular Biology, N.I.A.M.D. Using double fluorescent staining techniques the distribution of T-antigen content is being determined as a function of cell DNA content.

A study of the cytotoxicity of a therapeutically active protein (NSC-174951) has been conducted. This material has a very poor solubility and aggregation of the protein has been reported. We have extracted a cytotoxic component from this material which has a molecular weight of less than 10,000 on gel filtration chromatography. The chemical nature of this small molecular weight cytotoxic material remains to be determined.

A method was developed to evaluate the renal toxicity of NSC-118994 (Inox) in the mouse because renal toxicity is a limiting activity in clinical trials. This drug produces a marked acute decrease in urine volume and creatinine excretion in the mouse. Three other active analogs of NSC-118994 were evaluated for renal toxicity: (a) NSC-187757 (Cytox), (b) NSC-49928 (Adenox), and (c) NSC-68105 (Methox). All three were found to be just as toxic as the parent compound. None of them are suitable candidates for clinical trials to replace NSC-118994. This work was done in collaboration with the Laboratory of Experimental Chemotherapy, NCI.

Staff Publications:

Bono, Vincent H. Jr.: A Review of Mechanism of Action Studies on the Nitrosoureas. Cancer Chemotherapy Reports, 60: 29-36, 1976.

Bono, Vincent H. Jr.: Mechanism of Action Studies with DTIC. Cancer Chemotherapy Reports, 60: 141-148, 1976.

Bono, Vincent H. Jr., Byfield, John E., Hagemann, Ronald F., Lesher, Samuel, Valeriote, Frederick, and Vietti, Teresa: Cell Survival Systems for the Evaluation of x-ray and Drug Combinations. Cancer Chemotherapy Reports, Part 2, 5: 205-213, 1975.

Bono, Vincent H. Jr.: Cycle-Dependent Therapeutic Agents. Mammalian Cells: Probes and Problems, 168-178, 1975.

Canellakis, E.S., Bono, V.H. Jr., Bellantone, R.A., Krakow, J.S., and Fico, R.M.: Diacridines: Bifunctional Intercalators III. Definition of the General Site of Action. Biochimica et Biophysica Acta, 418: 300-314, 1976.

Jayaram, H.N., Cooney, D.A., Ryan, J.A., Neil G., Dion, R. L., and Bono, V.H. Jr.,:L-[αS,5S]-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid: A new amino acid antibiotic with the properties of an antagonist of L-glutamine. Cancer Chemotherapy Reports, 59: 481-492, 1975.

Wolpert-DeFilippes, Mary, Bono, Vincent H. Jr., Dion, R.L., and Johns, David G.: Initial Studies on Maytansine-Induced Metaphase Arrest in L1210 Murine Leukemia Cells. Biochemical Pharmacology, 24: 1735-1738, 1975.

Woolley, Paul V., Dion, Robert L, and Bono, Vincent H. Jr., : Effects of Tryptophan Deprivation on L1210 Cells in Culture. Cancer Research, 34: 1010-1014, 1974.

Woolley, Paul V., Dion, Robert L., Kohn, Kurt, and Bono, Vincent H. Jr., : Binding of 1-(2-Chloroethyl)-3-(Cyclohexyl)-1-Nitrosourea to L1210 Cell Nuclear Proteins. Cancer Research, 36: 1470-1474, 1976.

Contractor Publications:

Brockman, R.W., Shaddix, Sue C., Williams, Marilyn, Nelson, J. Arly, Rose, Lucy M., and Schabel, Frank M.: The Mechanism of Action of 3-Deazauridine in the Tumor Cells Sensitive and Resistant to Arabinosycytosine. Annals of New York Academy of Science, 225: 501-521, 1975.

Byfield, John E., Lee, Young C., Tu, Lorna, and Kulhanian, Fimi: Molecular Interactions of the Combined Effects of Bleomycin and X-Rays on Mammalian Cell Survival. Cancer Research, 36: 1138-1143, 1976.

Gosalvez, Mario, Garcia-Canero, R., Blanco, M., and Gurucharri-Lloyd, C.: Effects and Specificity of Anticancer Agents on the Respiration and Energy Metabolism of Tumor Cells.: Cancer Chemotherapy Reports, 60, 1976.

Gosalvez, Mario, Garcia-Canero, R., and Reinhold, Hubert : Delayed Pyridine Nucleotide Reoxidation Induced by the Anticancer Agent VM-26 as Measured in vivo and in situ by NADH Microfluorimetry. European Journal of Cancer, 11: 709-715, 1975.

Hill, Donald L.: Microsomal Metabolism of Triazenylimidazoles. Cancer Research 35: 3106-3110, 1975.

Liao, Lon-Lon, Horwitz, Susan B., Kupchan, S. Morris, and Grollman, Arthur : Mode of Action of the Antitumor Compound Bruceantin, An Inhibitor of Protein Synthesis. Molecular Pharmacology, 12: 167-176, 1976.

Nelson, J. Arly, Carpenter, Jean W., Rose, Lucy M., and Adamson, Doris J.: On the Mechanism of Action of 6-Thioguanine, 6-Mercaptopurine, and 8-Azaguanine. Cancer Research, 35: 2872-2878, 1975.

Nelson, J. Arly, Kuhns, Jane N., and Carpenter, Jean W.: Lack of Activity of β-2'-Deoxythioguanosine against Two Tumors Resistant to 6-Thioguanine. Cancer Research, 35: 1372-1374, 1975.

Nelson, J. Arly, and Rose, Lucy M.: A Simple Method for Heating Coiled Columns for High Pressure Chromtagraphy. Journal of Chromatography. 106: 188-190. 1975.

Nelson, J. Arly, Rose, Lucy, and Bennett, L. Lee, Jr.: Effects of 2-Amino-1,3, 4-thiadiazole on Ribonucleotide Pools of Leukemia L1210 Cells. Cancer Research, 36: 1375-1378, 1976.

Tobey, Robert A.: Different Drugs Arrest Cells at a Number of Distinct Stages in G_2. Nature, 254: 245-247, 1975.

Tobey, Robert A. and Crissman, Harry A.: Unique Techniques for Cell Cycle Analysis Utilizing Mithramycin and Flow Microfluorometry. Experimental Cell Research, 93: 235-239, 1975.

Tobey, Robert A., Crissman, Harry A., and Oka, Melvin S.: Arrested and Cycling CHO Cells as a Kinetic Model: Studies with Adriamycin. Cancer Chemotherapy Reports. (in press).

Tobey, Robert A., Oka, Melvin S., and Crissman, Harry A.: Differential Effects of Two Chemotherapeutic Agents, Streptozotocin, and Chlorozotocin, on the Mammalian Cell Cycle. European Journal of Cancer, 11: 433-441, 1975.

Wheeler, Glynn P., Bowdon, Bonnie J., and Struck, Robert F.: Carbamoylation of Amino Acids, Peptides, and Proteins by Nitrosoureas. Cancer Research, 35: 2974-2984, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUM
Z01-CM-03583-05 LMCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Modification of Cell Surfaces to Limit Cell Division and Increase Immunogenicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Muriel M. Lippman Senior Staff Fellow LMCB NCI

COOPERATING UNITS (if any)

Southern Research Institute (Birmingham, Alabama)

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

SUMMARY OF WORK (200 words or less - underline keywords)

Purified Macromomycin was evaluated on L1210, B16, and Lewis lung carcinoma and found to differ from the previously tested impure preparation in its activity against these tumors. This drug is presently undergoing toxicity and immunologic analysis and its mode of action as a chemoimmunotherapeutic agent is being further studied.

Project Description:

Objectives: 1. The project aim at exploiting those changes in cell surface which accompany neoplastic transformation with the object of finding cell division inhibitors which selectively bind to transformed cells.

2. In the case of manifest cancer, the immune response evoked by the malignant cells has failed to effect a complete rejection of the neoplastic cells. Studies are carried out with the aim of increasing the immunogenicity of the neoplastic cells and preventing some aspects of enhancement.

3. Application of cell surface molecules to cultured human cells is immediately relevant to clinical chemotherapy. To this end efforts were made to maintain human tumors in long term cultures.

Methods Employed:

Standard procedures for testing drugs on cultured cells and animals. Special protocols for chemotherapy in mice bearing transplantable tumors.

Major Findings:

Highly purified macromomycin (MCR) was found to be more active *in vivo* against Lewis lung tumors than the impure preparation but less active against i.p. B16. The purified material was considerably less stable but more active *in vitro* than the impure preparation.

Studies on the toxicity and immunologic aspects of the purified preparation are underway.

Proposed Course: None

Publications:

1. Coronetti,Emanuela and Lippman, Muriel M.: Increased Immunogenicity of TA3Ha Cells Treated with the Antitumor Antibiotic Macromomycin(B). Journal of the National Cancer Institute. (in press).

2. Lippman, Muriel M. and Mathews, Martin B.: Heparins: Varying Effects on Cell Proliferation In Vitro and Lack of Correlation with Anticoagulant Activity. Federation Proceedings (in press).

3. Lippman, Muriel M., Laster, W. Russell, Abbott, Betty J., Venditti, John M. and Baratta, Mario C.: Antitumor Activity of Macromomycin(B) (NSC-170105) Against Murine Leukemias, Melanoma, and Lung Carcinoma. Cancer Research, 35: 939-945, 1975.

SUMMARY REPORT OF THE EXPERIMENTAL THERAPEUTICS PROGRAM

NATIONAL CANCER INSTITUTE

NATIONAL INSTITUTES OF HEALTH

The Experimental Therapeutics Program (ETP) has responsibility for intramural and extramural research investigations concerned with the pharmacology, toxicology, and mode of action of cancer chemotherapeutic agents and other drugs used as single agents or in combined modalities. Studies in molecular biology and the development and use of biologic markers to assess tumor statis also constitute major areas of research for the Program. Thus, studies within the ETP cover a broad range of drug-host phenomena at the whole body, cellular and molecular levels.

The intramural laboratory research programs and contract operations are shared among the Office of the Associate Director and four laboratories: (1) Laboratory of Toxicology; (2) Laboratory of Chemical Pharmacology; (3) Laboratory of Molecular Pharmacology; and (4) Laboratory of Tumor Cell Biology.

Toxicological studies

In its dual responsibility for conducting basic toxicologic research and supervising a large contract operation, primarily by the Prime contract mechanism, for large animal protocol and special toxicity studies of antitumor agents, the Laboratory of Toxicology is a vital link in the chain of events which occur in the Division's drug development process. In this role the laboratory provides information describing the toxic manifestations of a broad spectrum of antitumor drugs in animal systems and develops experimental animal models for predicting organ-specific toxicities in man. It is hoped that the information will assist the clinician in establishing a safe starting dose for Phase I studies, in making intelligent predictions as to the adverse reactions which may occur in patients receiving various antitumor agents during early clinical trials and, in advising him on measures which may be taken for prevention or reversal of toxicity.

Highlighting some of the intramural laboratory research accomplishments during the past year: (1) Techniques were developed for the purification of L-asparagine synthetase to assist in studies of the biochemical toxicology of the oncolytic enzyme L-asparaginase and, in particular, to elucidate the mechanism of tumor resistance to this clinically effective carcinostatic agent. Homoserine-AMP of many chemicals tested and the metal ions of copper, colbalt, calcium and zinc were found to be effective inhibitors of L-asparagine synthetase. Although the latter was also shown to be of potential value as a biologic marker for tumor growth in rodents with Leukemia 5178Y/AR, its rapid clearance from serum may preclude its use as a diagnostic marker in man. (2) In the area of development of animal models, efforts are continuing to explore the potential of the rat for assessing the

cardiomyopathic effects of adriamycin and daunorubicin. A small animal species such as the rat is highly desirable to conduct these tests as in many cases, only limited quantities of drug are available during the early phases of the drug development process. Preliminary results indicate that the rat (F344) may also be a useful model for predicting the renal toxicity of cis-diamminedichloroplatinum and related compounds. (3) Drug metabolism studies have been conducted in some experimental animal species ranging from rodents to the non-human primate in continuing efforts to provide more predictive models of drug toxicity for man. In this regard, the in vitro hepatic and extrahepatic metabolism of the mini-pig was found to most closely resemble man. In a drug disposition study, a mathematical model was developed for cis-diamminedichloroplatinum which appears to be of potential usefulness for predicting the disposition of related compounds in various animal species and man.

The extramural effort of the laboratory largely involves the monitoring of protocol and special large animal toxicity studies being conducted by the Prime contract. This mechanism is now completing its second year of operation and is providing the Division with a more rapid and systematic accumulation of toxicity data for filing of an Investigational New Drug Application (INDA). The Prime contract has also provided some relief to the laboratory's investigators in allowing them to devote a greater portion of their efforts to intramural laboratory research projects.

Pharmacological studies

The primary responsibility of the Laboratory of Chemical Pharmacology is to maintain an intramural and contract research program for the development and use of analytical-biochemical methodology for studies of the pharmacological disposition, metabolism, and mechanism of action of antitumor agents in man, laboratory animals, and in vitro predictive systems. The long-term objective is to permit the design of more effective therapeutic dose schedules and drug combinations and other treatment modalities for the clinic.

A major part of the laboratory's effort continues to concern studies of the pharmacokinetics and biochemical pharmacology of folic acid antagonists, particularly methotrexate (MTX). The latter has elicited considerable interest among laboratory and clinical investigations during the past several years as a result of its expanding clinical use in high dose regimens with leucovorin rescue. Thymidine and carboxypeptidase-G are also being explored as rescue agents for high dose MTX. Using an in vitro bone marrow colony assay, it was demonstrated that MTX toxicity to mouse bone marrow resulting from inhibition of both purine and pyrimidine synthesis can be reversed non-competitively by thymidine and a purine source. With respect to rescue with calcium leucovorin, the in vitro assay resembles the bone marrow in vivo assay which involves measuring the degree of incorporation of nucleosides into DNA. Both assays indicate that the plasma concentration ratio between calcium leucovorin and MTX are critical in rescue efforts, with $5X10^{-7}M$ or less, MTX being a level at which leucovorin rescue can be discontinued without resulting in significant drug toxicity.

Pharmacologic disposition studies in laboratory animals were carried out on a variety of potentially useful new antitumor drugs. These included coralyne sulfoacetate (NSC-154890), inosine dialdehyde (NSC-118994), an acridine derivative (NSC-141549), 3-deazauridine (NSC-126849), spirohydantoin mustard (NSC-172112), and chlorozotocin (NSC-178248). In addition, the pharmacokinetics of gallium was studied in patients receiving the agent during Phase I clinical trials. In related studies, the absorption and distribution by lymphatic channels of a number of established antitumor agents were examined. Therapeutic advantage might be gained in treating lymphoid malignancies which metastasize *via* the lymphatics with agents selectively carried by the lymphatic channels.

Studies were also performed to determine the biochemical basis for the cytotoxicity of the acridine derivative, 3-deazauridine, and inosine dialdehyde. Of particular interest was the finding that 3-deazauridine is phosphorylated to a greater extent in tumor cells than in normal cells to the triphosphate which is not incorporated into cellular DNA or RNA, thus raising the possibility that cytotoxicity might be linked to inhibition of nucleic acid polymerases by the triphosphate form. The cytotoxicity of inosine dialdehyde may be related to its observed cross-linking between red blood cell membranes and plasma proteins.

Several pharmacological investigations have been conducted in the laboratories of the Office of the Associate Director (OAD) involving folate derivatives and the vinca alkaloid, vincristine. Distribution studies with 5-methyl-tetrahydrohomofolate did not provide any clues to explain its activity against antifolate-resistant variants of L1210 murine leukemia. Although N_{10}-formylfolate is a potent dihydrofolate reductase inhibitor *in vitro*, the drug did not have any significant antitumor effect *in vivo*. The availability of aromatically tritium labeled vincristine, and the development of a technique employing a high-pressure liquid chromatographic system for the purification and separation of this alkaloid from metabolic and degradation products, have permitted studies of its disposition in rats and dogs. Essentially, the drug is rapidly cleared from the plasma with an initial half-life of 15 minutes and a terminal phase of 75 minutes. The drug is excreted primarily in the feces *via* the bile and to a lesser extent in the urine mostly unmetabolized, although several minor potential metabolites have been detected but not identified in the plasma and tissue extracts.

The Membrane Transport Section in the OAD continued studies to quantify the transport of various drugs and molecules between blood, brain, and cerebrospinal fluid (CSF) of several species of animals for the purpose of developing methods to achieve more effective central nervous system concentrations of antitumor drugs. Experimental work and computor modeling of the delivery *via* CSF indicate that good delivery of the water soluble MTX and hydroxyurea to neural tissue adjacent to the CSF could be achieved but not for cytosine arabinoside, BCNU, and thiotepa. In general, intracarotid infusion was superior to intravenous infusion only when the time course of drug action did not exceed the period of intracarotid infusion and when the rate of drug uptake by brain (tumor), drug metabolism, degradation, and excretion was high.

Molecular biology and pharmacology studies

The Laboratory of Tumor Cell Biology maintains an intramural laboratory research program and a supporting contract operation to provide information on the molecular and physiological control mechanism in normal and neoplastic cells and to search for RNA tumor virus genomes and their products in human tumor tissues, particularly leukemia. Another important area of study involves the search for biochemical markers of minimal neoplastic disease and the development of useful microtests for the detection of such markers. Among the many new experimental observations made by the laboratory during the past year, probably most significant was the detection of the baboon oncornavirus related sequences in the DNA of the uncultured spleen of a patient with AML, by its hybridization to complementary DNA from the baboon related viral component of the putative human virus and to the RNA from baboon endogenous virus. This is the same patient whose cells were used for the isolation of the putative human virus reported in 1975. The baboon related sequences in DNA have been observed in the tissues of other patients with leukemia but the significance of this finding and its relation to human leukemia is being explored, particularly to determine whether RNA tumor viruses are involved in human leukemia.

Noteworthy in studies of potential biochemical markers for detection of a minimal number of tumor cells, was the demonstration of the presence of high levels of terminal deoxynucleotidyl transferase in cells of patients with ALL and some patients with CML in acute blastic phase of the disease. Such a marker may prove to be useful in following the course of therapy of leukemic patients.

During the past year, the Laboratory of Molecular Pharmacology's intramural research program was the first within the Division to be subject to an outside peer-review for scientific merit. It is gratifying to note that a team of distinguished scientists judged the quality of the laboratory's scientific program to be of high excellence, comparable to several such research groups at leading universities and other research institutions.

The primary emphasis of the laboratory's program was concerned with continuing studies on the damage, repair, and replication of DNA in relation to the effects of various alkylating antitumor agents on mammalian cells grown in culture. Key to these studies has been the development and application by the laboratory over the past 4 years of the alkaline elution analysis technique for assessing DNA damage. This relatively uncomplicated technique promises to be of major importance in studies related to the effects of a variety of chemotherapeutic agents or radiation on DNA damage and repair. The laboratory will explore broader application of the method in ensuing years.

Several of the observations made during the past year using the above and other techniques include: (1) Normal human cells (WI-38) were less sensitive than the SV-40 transformed (VA-13) human cells to the killing effects of BCNU, although no significant differences in the formation and disappearance of DNA breaks were seen between the two cell lines. (2) Prior exposure of

WI-38 cells to BCNU delayed the disappearance of DNA breaks produced by sub-lethal doses of x-ray as contrasted to further breakdown of DNA in VA-13 cells. (3) Bifunctional inter-strand crosslinking of DNA by BCNU and HN_2 was postulated to occur by a two-step reaction which does not require the presence of free drug in the media after initial exposure. In contrast to BCNU, crosslinks caused by HN_2 were repaired after removal of drug thus permitting survival of L1210 cells. The significance of these above observations as related to the cytotoxic and therapeutic effects of these alkylating agents is expected to become more apparent as work progresses.

Biologic markers program

Considerable progress was made during the past year in the Biologic Markers Program, particularly in the establishment of the specimen repository, analytical services unit, and the initial segments of the basic research facility at the Frederick Cancer Research Center. A Program Director has been hired by Litton at Frederick and the basic research plan was presented to the Division's Board of Scientific Counselors for review and comment which was highly favorable. It is expected that all units of the Biologic Markers Program will be completely functional by the latter part of 1976. The research program will be addressed to both the development of new and the improvement of existing specific tumor markers. A comprehensive study of potential cancer markers will be undertaken in model systems in order to obtain information about this genesis and biological significance.

ANNUAL REPORT OF THE OFFICE OF THE ASSOCIATE DIRECTOR

EXPERIMENTAL THERAPEUTICS PROGRAM

NATIONAL CANCER INSTITUTE

NATIONAL INSTITUTES OF HEALTH

July 1, 1975 -- June 30, 1976

Studies of the transport of various physiological and pharmacological substances between blood, brain, and cerebrospinal fluid (CSF) in several species of animals have been undertaken. Intravascular infusions, ventriculo-cisternal perfusions, lumbo-cisternal perfusions, ventricular injections, theoretical and mathematical analysis, plus computer modeling have been the methods generally employed.

Experimental work and computer modeling of the delivery, via the CSF, of five drugs - methotrexate, hydroxyurea, cytosine arabinoside, thiotepa, and BCNU - was done. MTX and hydroxyurea entered the adjacent neural tissue readily and moved by diffusion to deeper sites within the brain. The transport of MTX throughout the CNS could be enhanced by decreasing the rate of CSF production or turnover and by introducing MTX into both the ventricular and subarachnoid CSF. The penetration of the other three drugs into the tissue was limited by cellular uptake (cytosine arabinoside) or capillary loss (thiotepa and BCNU). Significant accumulation of these three agents did not occur at CNS sites more than 2mm from the CSF-tissue interface.

From our analysis of intracarotid and intravenous drug distribution to the CNS and bone marrow, we determined that an intracarotid infusion gives a significant advantage of increased brain drug delivery when the rate of carotid blood flow is low. With an intracarotid infusion markedly decreased bone marrow drug uptake occurs only when the rate of tissue drug metabolism or excretion is high ($t_{1/2}$ = 5 min).

Tracer antipyrine has been used on numerous occasions to measure regional cerebral blood flow. Our double indicator studies with cats show that sizeable errors result from the use of labeled antipyrine and that the magnitude of these errors varies with the rate of blood flow.

The movements of three neurotransmitter metabolites, 3-methoxy-4-hydroxy-phenylethyleneglycol (MHPG), 5-hydroxyindoleacetic acid (HIAA), and homo-vanillic acid (HVA), between CSF, spinal cord, and blood was studied. All three moved rapidly across cell membranes within the cord. MHPG exchanged quite rapidly across spinal capillaries, whereas HVA and HIAA passed from spinal tissue to blood at somewhat lower rates.

A choroid plexus papilloma was found in a shark, Squalus acanthias, and studied histologically. This is the first known report of an intracranial tumor among the cartilaginous fishes. CSF secretion studies in a child with

a large (72 gm) choroid plexus papilloma indicated a very elevated rate of fluid production with such a pathological condition. Work with an intracerebrally implanted choriocarcinoma tumor model in monkeys showed that the capillary permeability and distribution space of extracellular materials, such as inulin, are much larger in this tumor than in normal brain tissue; however these alterations did not extend much (1-2 mm) beyond the grossly visible edge of the tumor mass.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 03500-12 ETP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Distribution of Drugs Between Blood, Brain, and Cerebrospinal Fluid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any) Biometrics Br., NIMH; Lab. Cerebral Metab., NIMH; Lab. Tox DCT, NCI; Fogarty Int. Center; Dept. Neurol., Cornell Med. Sch.; Dept. Neurosu Univ. Western Ontario; Med. Computer Center, Univ. Missouri; Dept. Neurosurg., Univ. Minnesota; Dept. Neurology, Memorial Sloan-Kettering Cancer Center

LAB/BRANCH
Office of the Associate Director for Experimental Therapeutics Program

SECTION
Membrane Transport Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.5	4.5	3.0

SUMMARY OF WORK (200 words or less - underline keywords)

Our work indicates that reasonably good delivery of water-soluble drugs, such a methotrexate and hydroxyurea to neural tissue adjacent to the CSF can be obtained when optimal CSF infusion protocols are used; however the CSF route of administration does not provide adequate distribution of cytosine arabinoside, BCNU, and thiotepa to these CNS sites. Intracarotid chemotherapy appears to offer a significant advantage in terms of increased brain uptake of drug when carotid blood flow is low or of decreased systemic delivery (drug toxicity) whe the rate of metabolism and/or excretion is high. From experiments with various brain tumor systems, we have found that the amount of CSF produced within the ventricular system of a child with a choroid plexus papilloma was greatly increased and have defined some of the changes in brain transport characteristi caused by an intracranial tumor. We have demonstrated that the uses of anti-pyrine as an indicator of regional cerebral blood flow and of various neuro-transmitter metabolites concentrations in the CSF as markers of brain metaboli are in error. The first precise measurements of spinal cord to blood transport rates of three of these metabolites were also made.

PHS-6040
(12-75)

Project Description:

Objectives:

The objectives of this project are to quantify the transport of various materials of physiological and pharmacological importance between blood, neural tissue, and cerebrospinal fluid (CSF) and to study the effects of various pharmacological agents and pathological conditions upon the transport and metabolism of essential materials, e.g. amino acids, within the blood-brain-CSF system. It is the purpose of such studies to characterize the transport properties of the blood-brain-CSF system and to develop methods for obtaining more effective central nervous system (CNS) concentrations of antitumor drugs.

Methods Employed:

The method of ventriculocisternal perfusion of various radioactive materials and subsequent brain tissue sampling has been utilized in monkeys, dogs, and dogfish sharks for measurements of brain capillary and cellular permeability. Furthermore a spinal cord perfusion technique has been developed and employed for similar studies in the spinal cord. Studies of brain and CSF uptake and distribution of intravenously administered, radioactively labeled physiological and pharmacological materials have been undertaken in dogs, normal and tumor implanted monkeys, and elasmobranchs. An experimental system for measuring the rate of CSF absorption has been devised and used to assess the ability to absorb CSF in normal and experimentally altered animals. Computer modeling has been used to study drug distribution between blood, CNS, and CSF following simulated intravenous, intra-arterial, and intraventricular administration.

Major Findings:

Our experimental and computer-modeling studies of drug distribution have yielded the following observations. After intraventricular administration methotrexate (MTX) and hydroxyurea cross the ependyma and diffuse through the brain extracellular fluid. Only small amounts of these drugs are lost from the CNS by transcapillary exchange. When introduced into the CSF, cytosine arabinoside (AraC) enters the adjacent neural tissue and accumulates in the cells. This action limits the penetration of AraC into deeper tissue. Intraventricularly infused BCNU and thiotepa cross the ependyma and exchange rapidly across brain capillaries, thereby being removed from all but the immediate periventricular tissue. More detailed analysis of our MTX data plus that of other researchers indicated that MTX concentrations in brain following CSF injection can be increased by: a) reducing the rate of MTX clearance from the CSF, b) distributing MTX throughout the ventricular and subarachnoid CSF compartments so that diffusional uptake occurs from both surfaces, and c) multiple dose scheduling. Brain concentrations of MTX will be reduced by drug metabolism and transcapillary loss.

From our analysis of intra-arterial (intracarotid) and intravenous drug distribution to the brain and bone marrow (the presumed site of systemic toxicity), we have found certain situations where an arterial infusion seems to be superior to a venous infusion. If the rate of carotid blood flow is low or lowered, higher brain concentrations and exposures (concentration-time product) will occur with an intracarotid administration of drug. If the rate of drug metabolism is high, diminished drug delivery to the bone marrow will take place with an intracarotid infusion. For most other situations there is little advantage, in terms of increased brain and/or decreased systemic drug delivery, with intra-carotid chemotherapy.

In our studies of tracer antipyrine exchange between blood and brain, we concluded that: 1) the uptake of ^{14}C-antipyrine in most regions of normal cat brain is limited by capillary permeability as well as by blood flow; 2) significant errors, therefore, result from the use of tracer antipyrine as the indicator for the measurement of local cerebral blood flow; and 3) the measurement of cerebral blood flow in conditions where alterations of flow are being evaluated is hazardous since the magnitude of these errors is flow-dependent.

An examination of material exchange between the CSF and the brain and spinal cord of monkeys was made using our techniques of lumbo-cisternal and ventriculo-cisterno-lumbar perfusion. From this work we determined that extracellular-type compounds exchange readily between CSF and CNS and that the rates of extracellular movement in spinal and brain tissue are comparable. Spinal transport studies of three neurotransmitter metabolites, 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), 5-hydroxyindoleacetic acid (HIAA), and homovanillic acid (HVA), demonstrated that CSF levels of these materials cannot be used as reliable indicators of spinal cord neurotransmitter turnover. The exchange of MHPG across spinal capillaries was quite rapid (exchange $t_{1/2}$ = 8 min), whereas the movements of HIAA and HVA across these capillaries was somewhat slower ($t_{1/2}$ = 19-20 min). For the latter two compounds, the transcapillary exchange process was apparently unaffected by the inhibitor probenecid.

During the course of our comparative studies on lower vertebrates, a choroidal papilloma of the fourth ventricle choroid plexus of a mature male dogfish shark, Squalus acanthias, was found and described. This appears to be the first report of a CNS neoplasm in an elasmobranch. In another study the rate of CSF formation was determined in a two year old child before and after the removal of a 74 gm choroid plexus papilloma from the left lateral ventricle. Preoperatively, the CSF formation rate was 1.05 ml/min; postoperatively, it was reduced to 0.20 ml/min. These data demonstrate the overproduction of CSF by a human choroid plexus papilloma.

An implanted choriocarcinoma growing in the brains of monkeys was used as a brain tumor model for a study of the cerebral distribution of inulin and albumin. The results indicated that the permeability of the capillaries and the size of the extracellular space were much larger in the tumor than in

normal brain tissue and that the tissue's capillary permeability and extracellular space were normal within 1-2 mm of the visible boundary of the tumor.

Significance to Bio-medical Research and the Program of the Institute:

Tumors, both primary and metastatic, frequently develop in the brain, spinal cord, and meninges. Anti-tumor agents can be delivered to these tissues by the vascular and/or the cerebrospinal fluid systems; However, each of these two systems appears to have certain limitations as a means of effective drug delivery. Basic information concerning the transport physiology and movement of material into normal and neoplastic CNS and meningeal tissue from the blood and from the CSF is needed for the design and development of effective brain tumor chemotherapy protocols. Such information about blood-brain-CSF transport coupled with a knowledge of the physiochemical and pharmacological properties of specific antitumor drugs will enable the chemotherapist to choose the best route, manner, and timing for effective drug delivery to CNS and meningeal lesions. Basic knowledge of the transport of ions, metabolites, and other materials between blood, brain and CSF is essential to the understanding of the activities and functions of the central nervous system. Since our work is fundamental in nature, it is relevant to work being carried out by many other groups of bio-medical researchers, and important for an understanding of both normal and pathological brain and spinal cord processes. The type of work performed in our laboratory on this subject is, thus, meaningful and significant to the neurologist, the neuroncologist, the neurosurgeon, and the neuropathologist as well as the neurophysiologist, neurochemist, and neuropharmacologist.

Proposed Course:

1. Continue our studies of the transport of various physiological and pharmacological materials between blood, brain, and CSF and more precisely quantitate the permeability of brain capillary and cellular membranes.

2. Investigate further the formation, flow, and absorption of cerebrospinal fluid by and through CNS tissue.

3. Examine the effects of high and low dietary levels of vitamin A on CSF production, absorption, and pressure and on brain cell and capillary permeability of the rhesus monkey.

4. Determine the blood-spinal cord-CSF exchange parameters of various physiological and pharmacological agents.

5. Investigate, when appropriate fresh tissue is made available from either human or laboratory animal sources, the transport characteristics of various types of CNS tumors.

6. Study fluid and electrolyte flows under the pathological conditions of cerebral edema and hydrocephalus.

7. Investigate the effects of various transport inhibitors such as amiloride and phloretin on the blood-brain-CSF exchange of ions and amides in elasmobranchs and mammals.

Publications:

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2. Kessler, J.A., Fenstermacher, J.D., and Owens, E.S.: Spinal subarachnoid perfusion of rhesus monkeys. Am. J. Physiol. 230: 614-618, 1976.

3. Kessler, J.A., Fenstermacher, J.D., and Patlak, C.S.: 3-Methoxy-4-hydroxyphenylethyleneglycol (MHPG) transport from the spinal cord during spinal subarachnoid perfusion. Brain Res. 102: 131-141, 1976.

4. Kessler, J.A., Fenstermacher, J.D., and Patlak, C.S.: Homovanillic acid transport by the spinal cord. Neurology (in press).

5. Prieur, D.J., Fenstermacher, J.D., and Guarino, A.M.: A choroid plexus papilloma in an elasmobranch, Squalus acanthias. J. Natl. Cancer Inst. (in press).

6. Kessler, J.A., Patlak, C.S., and Fenstermacher, J.D.: Transport of 5-hydroxy-3-indoleacetic acid by spinal cord during subarachnoid perfusion. Brain Res. (in press).

7. Fenstermacher, J.D. and Cowles, A.L.: Theoretical limitations of intracarotid infusions in brain tumor chemotherapy. Cancer Treat. Rep. (in press).

8. Kessler, J.A., Gordon, E.K., Reid, J.L., and Kopin, I.J.: Homovanillic acid and 3-methoxy-4-hydroxyphenylethyleneglycol production by the monkey spinal cord. J. Neurochem. (in press).

9. Blasberg, R.G., Patlak, C.S., and Shapiro, W.R.: Distribution of methotrexate in the CSF and brain after intraventricular administration. Cancer Treat. Rep. (in press).

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12. Ausman, J.I., Levin, V.A., Brown, W.E., Fenstermacher, J.D., and Rall, D.P.: Brain tumor chemotherapy: pharmacologic principles derived from a monkey brain tumor model. J. Neurosurg. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03516-10 ETP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biochemical Pharmacology and Pharmacodynamics of Selected Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	M. C. Castle	Staff Fellow	ETP NCI
	D. A. Margileth	Clinical Associate	ETP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Associate Director for Experimental Therapeutics Program

SECTION

Office of the Associate Director for Experimental Therapeutics Program

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7	4	3

SUMMARY OF WORK (200 words or less - underline keywords)

This project is directed towards the acquisition of information in biochemistry, pharmacology, toxicology and chemotherapy related to experimental and established antitumor agents. When these data are available attempts can be made to optimize clinical therapy by developing animal pharmacokinetic models for application to the human situation. In addition basic phenomena related to cancer chemotherapy are also investigated in order to broaden the rational approach to the design of drugs, to understand better the interaction of drugs in vitro and in vivo and to develop a total pharmacodynamic profile of antitumor agents in addition to their intrinsic abilities to damage cells.

roject Description:

bjectives:

o investigate the biochemical pharmacology and pharmacodynamics of new or stablished antitumor agents to better define the important parameters elated to their chemotherapeutic activity.

ethods Employed:

tandard laboratory procedures were employed.

ajor Findings:

he two major areas of investigation in this project involve folate erivatives and vincristine.

he distribution of 5-CH_3-C^{14}-tetrahydrohomofolate has been investigated in ice. As was expected from the work of others with isolated enzymes, easurable amounts of C^{14}-carbon dioxide was excreted in the breath in normal ice. There was nothing remarkable about the distribution of the drug which ppeared to be excreted mainly unchanged in the urine and certainly nothing o explain its remarkable effect against antifolate-resistant variants of 1210. Work in other laboratories has demonstrated that the drug enters ells in vitro by a mechanism distinct from methotrexate and folate. lthough it appears that less than 1% of the dose is demethylated to give arbon dioxide, it will be extremely important to do distribution studies in umor-bearing animals as its specificity towards the antifolate resistant umors may well depend on the tumors ability to remove the methyl group and ive rise to a local and lethal concentration of tetrahydrohomofolate.

10-formylfolate was evaluated as an antileukemic agent against L1210 and ound to be devoid of all antileukemic activity and also without any effect n the incorporation of deoxyuridine formate and thymidine into DNA. It ould thus appear that in spite of its potent effect against dihydrofolate eductase in vitro this drug is incapable of producing any biologically ignificant effect in a tumor-bearing mouse.

he studies with vincristine have involved both clinical and preclinical rojects. The preclinical work has focused on the distribution of purified ritiated vincristine in rat and dog. In both rat and dog the drug was apidly cleared from the blood. The decay curve was biphasic in both animals ith $t_{1/2}$ of 15 minutes for the first phase and 75 minutes for the second hase. Twenty-five percent of the dose appeared in the bile within 30 inutes and approximately 15% of the label appears in the urine in 72 hours. he tissue levels were not remarkable except that this highly neurotoxic gent was not able to penetrate the brain in either species studied. Thin ayer chromatography of bile and urine revealed that most of the radio- ctivity is unchanged vincristine. In the plasma only 50% of the label is

unchanged vincristine while there are two other labeled compounds detected which have not been identified but which are probably closely related to the parent molecule. In the rat 85% of the injected dose is accounted for in urine and feces over 72 hours. High pressure liquid chromatography of tissue extracts etc. suggest that only a minor amount of the dose is present as metabolite(s). Rat-liver homogenates did not metabolize vincristine at all.

Studies of the uptake of vincristine by L1210 cells in vitro and the effects thereon of other antitumor agents which could be used in combination with it revealed that most of the other antitumor agents and antibiotics have no significant effect on the uptake of H^3-vincristine.

The closely related drug vinblastine and a potent new alkaloid, maytansine, did however significantly decrease the uptake of vincristine. Further studies showed that the inhibition of vincristine uptake by vinblastine is due to its effects both on transport and binding.

Clinical studies are presently underway to measure the physiological distribution of labeled vincristine in five patients. Another clinical observation may be related to vincristine's inclusion in many multimodality therapies. Two patients with acute lymphoblastic leukemia were given multimodality therapy including vincristine and went blind. There was no evidence of meningeal leukemia in these two patients and there were no visual effects in other patients on the same protocol.

Proposed Course of Project:

The project will continue to follow a course aimed at the acquisition of information to give a complete pharmacologic profile of antitumor agents. Basic phenomena relating to drug action and toxicity will also be investigated.

Publications:

1. Sieber, S.M., Mead, J.A.R., and Adamson, R.H.: The pharmacology of antitumor agents from higher plants. Cancer Treat. Rep. (in press).

2. Liss, R.H., Charest, M.C., and Mead, J.A.R.: Comparative ultrastructure of salivary glands from mice treated with cytosine arabinoside, cyclocytidine and fluorocyclocytidine. Cancer Treat. Rep. (in press).

3. Castle, M.C.: Isolation and quantitation of picomole quantities of digoxin, digitoxin and their metabolites by high-pressure liquid chromatography. J. Chromatogr. 115: 437-445, 1975.

4. Margileth, D.A., Poplack, D., Pizzo, P.A., and Leventhal, B.G.: Blindness during remission in acute lymphoblastic leukemia - a possible toxicity of multimodality therapy. Cancer (in press).

5. Oster, M.W., Margileth, D.A., Simon, R.W., and Leventhal, B.G.: Lack of correlation between lymphoblast size and prognosis in acute lymphocytic leukemia. Br. J. Haematol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06124-04 ETP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (60 characters or less)

Biologic Markers Program for Clinical Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. P. Waalkes	Senior Investigator	ETP NCI
OTHER:	D. C. Tormey	Head, Med. Breast Cancer Sec.	MO NCI
	R. H. Adamson	Chief, Lab. of Chem. Pharmacol.	LCP NCI
	D. Lakings	Visiting Fellow, IPA	LCP NCI

COOPERATING UNITS (if any)

NCI-Breast Cancer Service; Solid Tumor Service; Pediatric Oncology Service; Johns Hopkins Oncology Center; Mayo Clinic; Hoffmann LaRoche; Oak Ridge National Laboratory and University of Missouri

LAB/BRANCH

Office of Associate Director for Experimental Therapeutics Program

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

SUMMARY OF WORK (200 words or less - underline keywords)

Studies continue in an effort to develop new and better biologic markers for cancer. For those under evaluation, application has been and will continue to be made to specific clinical studies for consideration as to the eventual place each potential marker may have in the treatment of cancer. Investigations include specific protocol studies involving (1) patients with chemotherapy responsive disease undergoing experimental therapy, particularly breast cancer, (2) patients following surgery for cancer and (3) patients in remission following chemotherapy or radiotherapy. Ongoing evaluations include carcinoembryonic antigen, human chorionic gonadotropin, tRNA nucleosides, polyamines, β-aminoisobutyric acid, urinary hydroxy proline, serum protein bound carbohydrates and specific serum enzymes as possible sensitive indicators of metastatic tumor involvement of the liver.

Project No. ZO1 CM 06124-04

Project Description:

Objectives:

The objectives of this program are to develop and evaluate biologic markers which can be utilized as a means for following cancer patients during therapy, particularly chemotherapy, or during a disease free interval to evidence of recurrence. Multiple analytical methods are involved including high-pressure liquid chromatography, gas liquid chromatography, GC-mass spectrometry, amino acid analyzer, and radioimmunoassay. Specific materials as potential markers under evaluation include carcinoembryonic antigen, human chorionic gonadotropin, tRNA nucleosides, polyamines, β-aminoisobutyric acid, β-alanine, Regan's isoenzyme, serum fucose, sialic acid, urinary hydroxy proline/creatinine ratios, and β-glutamyltranspeptidase. Studies involve the determination of levels in advanced (broad spectrum) malignant disease as compared to normal and disease controls, and also specific neoplastic disease studies, e.g., markers in various stages of breast cancer, in metastatic lung cancer, in advanced gastrointestinal cancer, and in Burkitt's lymphoma. To date specific tRNA nucleosides and polyamines have been found elevated in the urine of greater than 50% of patients with several different solid tumor types. Three markers, carcinoembryonic antigen, human chorionic gonadotropin and N^2,N^2-dimethylguanosine have been found elevated in 97% of advanced breast cancer patients. tRNA nucleosides, polyamines, and β-aminoisobutyric acid/β-alanine ratio appear to be particularly useful for following patients with Burkitt's lymphoma. Polyamines, primarily spermine, are of distinct interest as possible markers for advanced colon carcinoma. Carcinoembryonic antigen, in preliminary studies, may be potentially useful in following patients with breast cancer. Bronchogenic carcinoma, as well as gastrointestinal malignancy, needs further evaluation. Continued developmental work is underway and more clinical studies projected. If successful, biologic markers may be of great assistance in evaluating patients with cancer during treatment, and in determining recurrence of disease following surgery, radiation or chemotherapy.

Contracts as Direct Extensions:

1. Oak Ridge National Laboratory - development of special instruments and related analytical techniques which can be used to measure specific potential markers. Work has progressed on tRNA nucleosides and on serum glycoproteins. (Contract No. NCI-CR-71-60; Budget (1975) = $85,000).

2. University of Missouri - development of methods, especially using such instruments as gas liquid chromatography and amino acid analyzer, for sensitive determination of potential markers and also, of practical methods, which can be used for routine analyses. Currently carring out specific studies on body fluid samples from patients in the various investigations underway. (Contract No. NIH-NO1-CM-12323; Budget (1975) = $140,000).

3. Johns Hopkins University - an independent but related project evaluating specific biosynthetic methods for labeling myeloma proteins in mouse models for determination of in vivo synthetic rate. The latter will be correlated with total tumor cell number. (Contract No. NO1-CM-43718; Budget (1975) = $38,000). Application of the technique for humans with this disease is under consideration.

4. University of Arizona - an independent project: (1) appraisal of a method for determining total tumor cell number for patients with multiple myeloma during the course of their illness and as a means for evaluating therapeutic response; (2) development of a staging system, based on above method (a) using pre-treatment values, to predict eventual outcome of disease as related to total tumor cell burden, treatment, and specific clinical parameters. (Contract No. NO1-CM-33713; Budget (1975) = $120,000).

Publications:

1. Tormey, D. C., Waalkes, T. P., Ahmann, D., Gehrke, C. W., Zumwalt, R. W., Snyder, J., and Hansen, H.: Biologic markers in breast cancer. I. Incidence of abnormalities of CEA, HCG, three polyamines, and three minor nucleosides. Cancer 35: 1095-1100, 1975.

2. Waalkes, T. P., Gehrke, C. W., Bleyer, W. A., Zumwalt, R. W., Olweny, C. L. M., Kuo, K. C., Lakings, D. B., and Jacobs, S.: Potential biologic markers in Burkitt's lymphoma. Cancer Chemother. Rep. 59: 721-727, 1975.

3. Levine, L., Waalkes, T. P., and Stolbach, L.: Serum levels of N^2,N^2-dimethylguanosine and pseudouridine as determined by radioimmunoassay for patients with malignancy. J. Natl. Cancer Inst. 54: 341-343, 1975.

4. Mrochek, J. E., Dinsmore, S. R., and Waalkes, T. P.: Liquid chromatography analysis of neutral carbohydrates in serum glycoproteins. Clin. Chem. 21: 1314-1320, 1975.

5. Makulu, D., and Waalkes, T. P.: Interaction between aromatic diamidines and nucleic acids. J. Natl. Cancer Inst. 54: 305-309, 1975.

6. Waalkes, T. P., Gehrke, C. W., Tormey, D. C., Zumwalt, R. W., Heiser, J., Lakings, D. B., Ahmann, D., and Moertel, C. G.: The urinary excretion of polyamines by patients with advanced cancer. Cancer Treat. Rep., in press.

7. Krolikowski, F. J., Reuter, K., Waalkes, T. P., and Adamson, R. H.: Serum sialic acid levels in lung cancer patients. Pharmacology, in press.

8. Lakings, D. B., Gehrke, C. W., and Waalkes, T. P.: Determination of TMS methylated nucleic acid bases in urine by gas-liquid chromatography. J. Chromatogr., in press.

9. Waalkes, T. P., Gehrke, C. W., Lakings, D. B., Zumwalt, R. W., Kuo, K. C., Jacobs, S. A., and Borek, E.: Brief communication: β-Aminoaciduria by patients with Burkitt's lymphoma. J. Natl. Cancer Inst., in press.

10. Waalkes, T. P., and Borek, E.: Biochemical assessment of the malignant status in man; aspects related to tRNA modification. Excerpta Medica, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06127-02 ETP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Modified Degradation Products of Normal Monkey Liver and Hepatoma tRNA

NAME, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. P. Waalkes	Senior Investigator	ETP NCI
OTHER:	D. B. Lakings	Visiting Fellow, IPA	LCP NCI
	R. H. Adamson	Chief, Lab. of Chem. Pharmacol.	LCP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Office of Associate Director for Experimental Therapeutics Program

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 1/2	1 1/4	1/4

SUMMARY OF WORK (200 words or less - underline keywords)

A specially designed column for high-pressure liquid anion chromatography with UV-absorption was used to compare the modified nucleosides and/or bases in tRNAs from normal monkey liver and hepatoma. In addition, degradation products found in urine e.g., β-aminoisotubyric acid were determined for respective normal and hepatoma monkeys as well as other associated excretion products, e.g., methylated nucleosides, pseudouridine and polyamines. These studies are being carried out to gain information as to changes in tRNA associated with tumors and the reflection of these changes as noted by their urinary excretion products and levels.

PHS-6040

Project Description:

Objectives:

tRNAs were isolated and purified from normal monkey liver and hepatomas induced by N-diethylnitrosoamine. Urine samples were also obtained prior to death of the monkeys. The tRNAs are being degraded and base or nucleoside analyses carried out depending on the mode of hydrolysis, by acid or enzymatically. By means of a special nucleoside and free base high-pressure anion exchange liquid automated chromatography with UV-absorption for measurement, the free bases and/or the nucleosides are being analyzed in the tRNA and the modified (primarily methylated) nucleosides quantitated in the urine for correlation. β-aminoisobutyric acid and β-alanine as urine excretion products of pyrimidines as well as polyamines are also being analyzed in the urine. Comparisons between normal and hepatoma monkeys are being made. To date elevated amounts of modified nucleosides, polyamines and β-amino isobutyric acid, have been found in the hepatoma monkey urines. Little, if any, increase in methylated nucleic acid bases in the hepatoma tRNA has been found except for thymine, secondary to methylation of uracil. By means of pseudouridine levels in tRNA and in urine, increased tRNA turnover for the hepatoma tRNA appears evident.

Publications:

Lakings, D. B., Gehrke, C. W., and Waalkes, T. P.: Determination of TMS methylated nucleic acid bases in urine by gas-liquid chromatography. J. Chromatogr., in press.

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06135-01 ETP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Tumor Cell and Biologic Marker Kinetics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. B. Woo	Senior Staff Fellow	ETP NCI
OTHER:	T. P. Waalkes	Senior Investigator	ETP NCI
	V. T. Oliverio	Associate Director	ETP NCI
	V. H. Bono	Head, Mol. Biol. and Methods Develop. Section	DR&D NCI

COOPERATING UNITS (if any)

Johns Hopkins Oncology Center, Johns Hopkins University School of Medicine, Drug Research and Development Program, DCT, NCI

LAB/BRANCH

Office of Associate Director for Experimental Therapeutics Program, NCI

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	0.3

SUMMARY OF WORK (200 words or less - underline keywords)

This project is designed to investigate the kinetic behavior of tumor cell-biologic marker interactions before and during therapy, utilizing both experimental and analytical (computer simulation) techniques, including the following: 1) the quantitative analysis of the dynamics of multiple marker distribution patterns for patients with advanced malignancies before and after therapy and its utility to assess the disease course of the patients during therapy; 2) to estimate, by measuring the concentrations of the biologic markers in the body fluids of the patients, the number of tumor cells via a quantitative model that integrates cell kinetics with the synthesis of biologic markers by tumors; 3) the time-course behavior of antitumor drug perturbation of the cell age, cell size, and DNA content distributions in relation to the synthesis of biologic marker and implications for the optimal design of antitumor drug schedules for cancer therapy.

CEA alone. Twelve marker measurements excluding CEA resulted in R^2=0.54 ($P<0.05$), suggesting that the influence of CEA on other marker measurements is negligible.

In patients with carcinoma of the colon and rectum, CEA plasma levels in the pre-treatment samples were elevated for 90% of both male and female patients. The three nucleosides were elevated for more than 35% of both male and female patients, and three polyamines for 35% of male patients and more than 15% of female patients, respectively. Percent of incidence of one or more marker abnormalities was about 98%. The correlations of each individual marker with disease status were fairly good with four marker measurements for female patients, including the pre-treatment measurements of CEA and Spd and the post-treatment measurements of Pu and Spd, while those for male patients were poor. Due to relatively small number of patients, the multivariate regression equation with 14 marker measurements cannot predict the disease status with statistical significance; with 12 marker measurements excluding the pre-treatment measurement of ψ and the post-treatment measurement of m'^{I} the multiple correlation coefficient (R^2) of 0.34 ($P<0.25$) was achieved for male patients and R^2=0.53 ($P<0.25$) for female patients, respectively. The study also revealed that CEA was dominant in female patients, while the post-treatment measurements of Pu and Sp were more significant in predicting the disease status of male patients.

2. Tumor Cell-Biologic Marker Kinetics. Further improvement on a cell cycle stage-specific model integrated with reaction kinetics of biologic marker synthesis was made in reference to the L1210 tumor-polyamine system. The kinetic behavior of key parameters associated with reaction kinetics was examined in relation to variations in cell kinetic parameters, mainly cell loss rate, which indicates that putrescine level varies more sensitively than spermidine and spermine levels.

A major effort was initiated to construct the biologic marker content distribution by a quantitative model that integrates cell kinetics with the synthesis of a biologic marker by tumor cells in the cell cycle. The cell age distribution was extracted from the DNA content distribution (and the cell size distribution) measured by a flow microfluorometric method; and then, it was combined with the rate of marker synthesis in cell cycle, which results in a biologic marker content distribution that enables one to estimate the number of tumor cells by measuring the marker concentration. Any variation in cell number due to therapy was correlated with the corresponding change in the synthetic rate of the marker. The cell age distribution was successfully generated for L1210 leukemic cells and the marker content distribution integrating the kinetic relationship between L1210 leukemic cells and polyamines is being carried out.

The cell age distribution was extracted from the cell size distribution measured by 1-g sucrose gradient method and then combined with polyamine content per cell in cell cycle to determine the number of tumor cells in terms of cellular content of polyamines; the simulation result compares favorably with available data.

3. Tumor-Growth Kinetics. The growth kinetics of L1210 leukemic cells in the spleen in relation to the ascites tumor growth was investigated by the cell cycle stage-specific model, which predicts the number of cells in the spleen based on the ascites tumor cells and correlates it with observed spleen weight.

An analysis of cytokinetic data on Burkitt's lymphoma by the cell cycle stage-specific model was carried out which specifically characterizes the growth fraction, mitotic index, labeling index, and growth rate during the entire growth period. This study is designed to investigate the tumor growth and regression of Burkitt's lymphoma via the measurement of the concentration of biologic markers (polyamines, nucleosides, and β-aminoisobutyric acid) in patients with Burkitt's lymphoma during and after therapy.

The proliferation and cell cycle kinetics of L1210 leukemic cell population was studied by the discrete-time kinetic model that generates the cell age distribution and subsequently predicts cell size and DNA content distribution which agree closely with available data. Other cell kinetic parameters characterized include growth fraction, cell loss rate, and cell cycle times in each different cycle phase during the proliferation of L1210 leukemic cell

The kinetic features of the DNA content distribution measured by flow microfluorometry was studied with respect to various patterns of DNA synthesis rates combined with different values of the coefficient of variation of DNA content. Detailed insights into correlation between the DNA content distribution and the cell age distribution were obtained.

The effects of antitumor drugs on the cell cycle is continuing, mainly in terms of the antitumor drug perturbation of DNA content distribution and the corresponding repression (inhibitory action) exerted upon the DNA synthesis rate by which the different modes of drug actions, such as cell kill and the progression delay of cells through the cell cycle phases are quantitatively related to possible changes in the cell kinetic parameters, the cell cycle time, the rate of cell loss, and the growth fraction.

Significance to Biomedical Research and the Program of the Institute:

The usual clinical means for measuring tumor progression or response to therapy, using individual isolated lesions, as a crude estimate may at times be in error in reference to total tumor burden. Consequently the use of such measurements as absolute could reduce the incidence of correlation with marker values. The quantitative analysis of multiple marker distribution patterns demonstrate the utility of the approach and suggest the potential value multiple marker analysis could have in aiding the assessment of the individual patient undergoing therapy. Although a high degree of statistical significance to assess the interdependence of the various markers would require a rather large sample size, the approach developed still provides a framework useful for characterizing different combinations of the markers in predicting and monitoring the clinical course of the disease.

A quantitative relationship between tumor cells and a marker, with reference to the kinetics of L1210 leukemic cells and polyamines, is of importance in evaluating therapy and designing therapy schedules. Such relationship may be developed for other types of tumor cells and corresponding markers, which will require different forms of functional relationship for the kinetic parameters representing the control exerted by markers on tumor cell proliferation.

An analytical scheme developed for extracting cell age distribution during tumor cell proliferation from either DNA content distribution or cell size distribution experimentally measured is of considerable value as the cell age distribution provides the important baseline kinetic parameter characterizing tumor cell kinetics. Combining the cell age distribution with the rate of biologic marker synthesis in cell cycle, one can generate the biologic marker content distribution by which the tumor cell number can be estimated in terms of the marker concentration. Further analysis of the reaction kinetics of biologic marker synthesis during cell cycle should provide new insight into the time-course behavior of cell kinetic parameters, such as cell loss, rate and growth fraction characterized in terms of the levels of marker concentration.

The cell cycle stage-specific model previously developed for the growth kinetics of experimental tumor systems was further utilized to determine the proliferation kinetics of Burkitt's lymphoma. One of the practical values of the model would be that it is readily expanded to integrate various metabolic reactions occurring in each cycle phase (as demonstrated by the L1210 tumor-polyamine system), including the known biochemical pathways, into the model and their perturbation resulted from administration of drugs. The time-course of a number of the potential markers for Burkitt's lymphoma is presently under investigation.

Proposed Course:

1. Efforts will be continued to investigate the optimal sequence and number of multiple marker measurements in predicting the clinical course of the patients with various forms of advanced cancer, and to characterize the dynamic patterns of multiple marker distribution by which different forms of cancer could be screened. Such information will be utilized in designing by use of systems modeling techniques frameworks for the optimal therapeutic schedules for patients by monitoring changes over time in each individual marker.

2. Various types of the rates of marker syntheses experimentally illustrated or predicted in the cell cycle are combined with the cell age distribution to analyze and examine the nature of different marker content distributions and its possible correlation with the kinetic behaviors of other cell kinetic parameters. The marker content distribution will be further evaluated to enable an assessment to be made on tumor regression and progression by measuring the concentration of markers in the body fluids from patients with cancer.

3. The dynamics of tumor-drug interactions are expressed in terms of perturbation by antitumor agents on DNA contents of cells in the individual stages of the cell cycle while taking into account the effects of cell cycle and pharmacodynamics. Therapeutic responses of antitumor drugs on the DNA content distribution will be implemented in the kinetic behavior of the discrete-time model previously developed by formulating a subroutine for drug pharmacodynamics, and also by taking into account the drug effects of cell kill and the progression delay of cells. This study will be carried out with the L1210 leukemic-polyamine system under chemotherapy with ara-C, MTX and BCNU.

4. Experimental study will be designed to find out specifically in tissue culture the kinetic relationship between the levels of polyamines, β-amino isobutyric acid, and nucleosides in growing cultures and changes in cell numbers as well as key cell kinetic parameters with time. Both normal cells and neoplastic cells are considered, and then kinetic behaviors are compared. The results obtained from this study will be integrated with the marker content distribution and eventually evaluated in terms of the marker concentrations in body fluids to estimate the tumor burden in the patients with advanced malignancy.

Publications:

1. Kim, M., Bahrami, K., and Woo, K. B.: Mathematical description and analysis of cell cycle kinetics and the application to Ehrlich ascites tumor. J. Theo. Biol. 50: 437-459, 1975.

2. Woo, K. B., Brenkus, L. M., and Wiig, K. M.: Analysis of the effects of antitumor drugs on cell cycle kinetics. Cancer Chemother. Rep. 59: 847-860, 1975.

3. Woo, K. B., Wiig, K. M., and Brenkus, L. M.: Variation of cell kinetic parameters in relation to the growth rate of Ehrlich ascites tumor. Cell Tissue Kinet. 8: 387-390, 1975.

4. Brenkus, L. M., Wiig, K. M., and Woo, K. B.: A mathematical analysis of tumor growth kinetics. Math. Biosci., in press.

ANNUAL REPORT OF THE LABORATORY OF TOXICOLOGY

NATIONAL CANCER INSTITUTE

NATIONAL INSTITUTES OF HEALTH

July 1, 1975 to June 30, 1976

I. Introduction

The Laboratory of Toxicology continues in its dual responsibilities for conducting basic research and for the supervision of large animal toxicologic studies of antineoplastic agents prior to their introduction into man. This important latter role has actually been carried out in the past by a rather diffuse contract mechanism, with the Laboratory of Toxicology retaining direct responsibility to supervise these studies, summarize the information, and aid in the submission of an INDA to the Food and Drug Administration before a Phase I Clinical Trial is initiated. In February of 1974, the Division awarded a prime contract, wherein the responsibility for these studies is shared by the Prime Contractor (Battelle Memorial Institute) and this Laboratory. Undoubtedly this Prime Contract made available more time for the inhouse staff to more properly pursue some important aspects of research into toxicological mechanisms of action of antineoplastic agents. For the next fiscal year the Laboratory is committed to two major goals: 1) to continue the improvement of the inhouse staff efforts in the area of basic research; and 2) to cooperate with the Prime Contractor in maintaining the good technical momentum it started during its first two contract years. It is felt that with the continuing limitations of staff, it would be unrealistic to project any additional goals. On the other hand, if staff conditions are improved it is the strong desire of the Laboratory to define and support research in the area of further delineating mechanisms of toxicity of anticancer agents with the hope of proposing remedies for these toxic actions.

Attempts to increase the level of excellence of the senior staff have been helped, on the one hand, by the Prime Contract relieving them of the daily supervisory chores which drained on their time. On the other hand, the failure to replace the 20% loss of positions, particularly in the area of support functions, which has occurred over the past three to four years in this Laboratory, continues to be a major detriment to growth. Particularly devastating this year has been a 100% turnover in the Laboratory's secretarial positions. More importantly than the turnover, there has been less than minimal secretarial support available to the Laboratory for more than six months of the past year. This was caused by administrative and personnel staff squabbles about matters such as: 1) whether we should be allowed to retain a WAE slot that was formerly a permanent position. It took three months to resolve this problem and the position was vacant during this period. 2) whether we would be allowed to retain a Laboratory Chief's secretarial position at the previously billeted GS-6 level. It took nearly six months to resolve this one. The position remained vacant during this time. These efforts to deny, even temporarily, this kind of support, cost the

Laboratory dearly in terms of getting Prime Contract correspondence completed in a timely fashion and in terms of publication rate. For example, some award fees of thousands of dollars were held up more than 100 days beyond the allowed 45 days. In the latter case, the Laboratory had been publishing about 30 papers per year over the past few years, and this rate dropped to about 24 papers this fiscal year. When senior investigators had to wait more than a month to get their research results typed for publication, they became understandably reluctant to prepare many manuscripts. The major way the 24 papers were published was by a combination of secretarial support from investigator's wives and by using GS-9-11 technicians, etc. to do typing. It is my understanding that such utilization of GS-9-11's is not considered by management to be "cost effective". It remains to be seen if anything is done about this sort of situation that is a hindrance to the growth of any Laboratory. It is our belief that without growth, there can be only stagnation. In a desperate effort to infuse some "new blood" into this potentially stagnant situation, we have recently entered into a new area for us, i.e., recruitment of foreign fellows via mechanisms which do not count against personnel ceilings. Of course, such individuals are not expected to help with program activities of the Laboratory, but rather it is hoped that their presence will help in the basic research areas and in improving the sagging morale of the senior staff. While two Fogarty Fellows joined the Laboratory this year, it is too early to evaluate their impact. For the third year in a row, some senior investigators have sustained superior research efforts, in the face of unrestored slots, only by heroic personal efforts. It was predicted in the annual report of two years ago, that it was "unfair and unhealthy to expect this to continue." In all honesty it must be admitted that there is evidence of continued attenuation of these heroic personal efforts. For the second year in a row, we have had to prematurely terminate some projects due to staffing limitations. The management cry to "establish new priorities" but "within present resources" carries with it grave risks. The risk that you may terminate a highly worthwhile project because the potential is not currently recognized. Setting priorities in biomedical science simply has not yet achieved the fine state of art as found in the engineering and technological areas. In contemporary biomedical science, setting priorities is guess work and no matter how much information you put into a "guess", you are going to be wrong a high percentage of the time. We pray we have not terminated or held in abeyance the wrong projects. While we have emphasized the positive aspects of the Prime Contract, it must be recognized that some features of this effort have been unpredictable and represented a greater time burden for some individuals than had been anticipated. For example, it must be pointed out that the Prime Contract takes a different kind of effort because of its "incentive" features. In other words, because the contractor is receiving a portion of his fee from this mechanism, extensive documentation is required to justify the award or denial of fee. It has been very difficult to structure the roles of the members of the Laboratory in this award-fee situation. It has been recommended that there be no over-lapping roles between the persons requesting the work and the individuals recommending the award of funds as judged by performance of this same work. Thus three categories of personnel are

required for proper operation of the Prime Contract: 1) compound officers, 2) contract monitors and 3) evaluators. Hence, it became obvious this year, that with the Laboratory down to five senior staff slots, the requirements that the three roles not overlap was impossible to meet. Therefore, we were obliged to functionally delete two contract monitors. The position of evaluator should be at the professional level but is being accomplished by a technician. We continue to insist, as we have in the past annual reports, that the supervision of the contractors' research activities be done by members of the scientific staff who have considerable first hand knowledge about the research problems involved with the use of our often uniquely toxic antineoplastic agents. If the internal staff can not maintain and grow in this kind of knowledge, then some important observations by contractors can not be properly assessed. For example, while some contract effort is being expended in defining the cardiac toxicity of Adriamycin, the possible involvement of other organs which may have primary or secondary etiologic contributions has been investigated. Thus alterations have been demonstrated by the inhouse staff implicating kidney, bone, intestine, and liver as possibly contributory to the cardiotoxicity of this agent. The degree of emphasis of basic research was a turn up a new road as far as the Laboratory was concerned and during this fourth year of such a policy it would seem that considering the personnel cuts, some progress continues. Evidence for this will be seen in the individual reports following, where it will be particularly noted that some projects have been brought to logical conclusions and submitted for publication.

II. Internal Research Programs

The independent projects of the senior staff members are primarily directed toward attempting to understand the actions of antineoplastic agents, especially those actions which manifest themselves as some form of toxicity. It is only through an understanding of such actions that we will be able to advise clinicians intelligently as to what toxicity to expect, and when toxicity does occur, what steps may be taken to reverse it.

A. Biochemistry Unit: In the past year, the Biochemistry Unit has continued its involvement with several projects related to the biochemical pharmacologic and toxicologic properties of selected oncolytic enzymes. The major rationale for the heavy emphasis in this area has been that the resistance to L-asparaginase appears to correlate with the presence of elevated activity of L-asparagine synthetase in the tumor cell. Interruption of the synthesis of L-asparagine appears to be one method to render such cells sensitive once again to L-asparaginase therapy. In addition to the obvious therapeutic potential of such manipulations of enzymes, substrates, and inhibitors, these efforts are of profound biochemical significance. While the present therapeutic applicability of oncolytic enzymes is not widespread, these ongoing studies focus on the important basic concepts of the mechanisms of tumor-resistance, a phenomenon that has led to many drug failures in the clinic. In order to conduct an indepth biochemical and kinetic examination of these concepts,

it is imperative that purified enzymes be available. Thus, in the past years, emphasis has been placed on purification of L-asparagine synthetase. Toward this end, a variety of classical and contemporary purification techniques have been employed. At the present stage, about a one thousand-fold purification of the crude homogenate has been achieved. In addition to the wide spectrum of inhibitors of L-asparagine synthetase which were discussed in last year's annual report, present investigations have centered around the use of inhibitors such as metallic ions; it was found that, in addition to copper and cobalt, some endogenous ions such as calcium and zinc significantly inhibited the synthesis of L-asparagine in vitro. Of one hundred chemicals tested, homoserine-AMP was found to be an effective inhibitor of L-asparagine synthetase, the tandem pair enzyme of L-asparaginase. L-Asparagine synthetase was found to be of value as a biologic marker of neoplasia. Hence, this enzyme was found to appear in the serum seven days after implantation of Leukemia 5178Y/AR (L-asparaginase resistant). Furthermore, the levels of this enzyme in serum increased as the tumor grew and metastasized. Like many other tissue-specific enzymes (e.g., creatine phosphokinase (CPK) from cardiac and skeletal muscle), this enzyme was found to be rather rapidly cleared from the serum with a half-life of about one hour. The obvious extension of these studies to clinical serum samples has been initiated with the cooperation of two clinical groups. A sensitive assay for such samples has been developed but of 100 sera tested, there was no evidence of L-asparagine synthetase activity. One final area that the Biochemistry Unit continues to be involved with, is the development of new analytical methodology. For example, in studying the influence of Streptozotocin on the incorporation of [^{3}H]thymidine into DNA, in studies of the alkylation of DNA by Streptozotocin and in other studies involving the possible use of [^{14}C]formate as a marker of protein synthesis, a number of chromatographic, fluorometric, radiochemical and spectrophotometric methods have been devised.

Other ongoing studies in this area resulted from this unit's extensive experience with the metabolism of amino acids and thus a collaborative study was begun to identify the metabolic pathways followed by DL-alanosine (NSC-143,647). This group has discovered that DL-alanosine is a substrate for amino acid oxidases and that it also is readily transamidated. It now appears that this agent is metabolized first to α-keto-alanosine, which is then decarboxylated and finally it enters the citric acid cycle.

B. Comparative Pathology Unit: The primary research efforts of this group have been in the areas of elucidating pathologic effects of antineoplastic agents and in defining animal models of human diseases. The studies of pathogenesis of adriamycin-induced cardiomyopathy in rabbits have made available to the Drug Development Program a useful animal model for assessment of the relative cardiotoxicity of adriamycin analogues. The Comparative Pathology Unit has provided the leadership in the development of this rabbit model. A realistic animal model now is available for comparative toxicologic testing of a series of analogues. It is hoped that by such testing of analogues of adriamycin that those which have similar or higher cardiotoxic potential than the parent drug

will not be employed in patients. Some adriamycin-analogue testing may have to be done on a smaller animal model because of very limited drug quantities. Therefore, either independently or in collaboration with others, this Unit is continuing on the rat as an additional model for prediction of cardiotoxicity. To date however, of all the species tested only the rabbit and the monkey appear to develop a syndrome of congestive heart failure, the major dose-limiting toxicity in patients receiving adriamycin. The finding of calcium accumulation in myocardia of adriamycin-treated rabbits suggests that myocardial membrane permeability is altered. As was reported last year, adriamycin is toxic to several organ systems, other that the heart, e.g., the lymphoreticular, digestive, urinary, skeletal and integumentary systems. During this past year, the muscular system was added to this growing list because in addition to occasional histologic lesions, significant decreases of high energy phosphate compounds (glucose-6-phosphate, ATP and creatine phosphate) were seen in several skeletal muscles (diaphragm, masseter and quadriceps femoris) but such decreases were not seen in cardiac muscle. Hence it would appear that either adriamycin gains more access to skeletal than cardiac muscle or that the former muscle has high energy phosphate reserves that are more labile than those in heart muscle. In another target-organ program, this Unit investigated the pathologic effects of antineoplastic platinum analogues on the kidney. Since early results using the mouse were inconsistent, the rat was tested as an alternative model. Preliminary results using six platinum containing compounds, indicate the F344 rat is a sensitive predictor of this toxicity. The systematic evaluation of clinical and autopsy records from cancer patients who have received anticancer therapy in the Clinical Center continues. To date, five hundred autopsy records have been computerized and are available in a format suitable for querying. For example, it is now relatively simple to conduct a computer scan of these autopsy records with a question regarding the possible incidence of antineoplastic therapy-related lesions associated with a specific modality (i.e., x-ray, surgery, chemotherapy or any combination thereof).

C. Drug Interactions Unit: The major reason for the existence of this Unit is because of the recently recognized fact that because patients often receive several drugs simultaneously, the combination may result in an undesirable effect due to what is generally categorized as "drug-drug interactions". This is particularly a problem in patients with neoplastic disease, since they frequently receive a multitude of drugs which may alter the absorption, binding, distribution, excretion, metabolism or pharmacokinetics of the anticancer agent. Hence, methodology in this Unit has been developed and applied in a number of areas using a variety of antineoplastic agents and other drugs. The principal activities of this Unit are in the areas of drug metabolism and drug transport. Such activities are of importance not only in defining which parameters lessen drug toxicity, e.g., microsomal induction, but also in understanding factors increasing toxicity. These studies recently have achieved more importance because of the evidence gathered over the past few years that drug biotransformation, leading to irreversible binding to tissue molecules, is a major parameter associated with toxicity. More and more citations in the recent literature are offering evidence that relatively inactive

drugs can be activated in vivo to proximate toxicants. A major emphasis of this research group has continued in the area of species differences in drug metabolism. These studies are important since any drug development program is always seeking to discover the animal which metabolizes drugs in a manner most similar to man. Despite several years of lip service to this important concept, it turns out that very few investigative groups have used similar methodology in assessing the ability of a wide variety of species to metabolize drugs by both hepatic and extrahepatic organs. Hence, in the past year, this group has compared on a very equitable basis the in vitro drug metabolizing capability of hepatic and extrahepatic enzymes in the mouse, rat, hamster, guinea pig, rabbit, Tupaia (a primitive primate species), mini-pig, Squirrel monkey and Rhesus monkey. Part of the reason for undertaking these studies is to get some information on which species might most closely resemble not only man, but also, the Rhesus monkey. This latter species is becoming increasingly scarce and it is felt necessary to try to define a species as a possible replacement if this shortage continues. At present, there is no information upon which such a decision can be made. Preliminary evidence suggests that of all nine species tested, the in vitro metabolism of the mini-pig seems to be most similar to the human. This Unit also has been active in studying the disposition of cis-diamminedichloro platinum in dogs, rats and dogfish shark. The first phase of these studies has been completed and accepted for publication. It has been found that a combination of disposition and flow-limited pharmacokinetic models provides a method for the rapid accrual of information on such compounds. For example, in dogs the plasma levels decayed with an initial half-time of one hour and a final half-time of about 42 hours. Similar results have been seen in the rat studies. Remarkable changes (i.e., thirty-fold) occurred in the clearance of this compound and appear to coincide with similar changes which were observed in the in vitro binding of this platinum analogue to plasma protein. Such evidence is suggestive of a spontaneous process, perhaps involving exchanges of the amino and/or chloride portions of the molecule. Similar distribution studies conducted in the dogfish shark suggest that exchanges involving the chlorine moiety were more important because in this aquatic species, which has about two to three times greater plasma chloride ion concentration than does the dog, such an exchange of chloride from the drug compared with plasma was inhibited about three-fold. In the dog, liver, kidney, ovary and uterus particularly accumulate the drug. Further studies were completed to assess the simultaneous pharmacokinetics and toxicity of this agent because of the profound differences seen in the clearance of the drug. It was found that the major changes in the clearance were not due to renal toxicity, but rather, represent true pharmacokinetic changes. Studies have been conducted on drug transport, in general, and in particular biliary, renal and peritoneal transport. The primary model for the biliary and renal studies over the past few years has been the dogfish shark. The advantage of this species seems to be that it is capable of accomplishing transport and metabolic changes "in slow motion". This has proven to be of particular advantage in the study of antineoplastic drugs which are metabolized and transported sometimes at rates too rapid to accurately measure. Studies on biliary transport are important in

this field, first in understanding which properties of drugs are important for biliary transport. It is hoped that such studies will provide a basis for selecting drugs which follow the biliary route as a major route of excretion and thus might be suitably employed in the chemotherapy of gastrointestinal cancers. These studies are desperately needed because of our relatively poor record of ameliorating cancers in this organ system. Study of hepatic excretion also is important to the pharmacologist in characterizing the metabolism and clearance of a drug by the body. For example, a drug which undergoes enterohepatic circulation can remain in the body for considerably longer times than might otherwise be expected. When this does occur, one must alter dose-scheduling and anticipate higher levels of toxicity. In a third area of study of the influence of drugs on biliary flow, it is of interest to develop methodology to accelerate these processes when it is desired to rapidly remove a drug via this mechanism. For example, it has been shown that for many drugs there is an accelerated biliary transport after prolonged treatment with the sedative phenobarbital.

D. Collaborative Efforts With Other NCI Units: Over the past few years, the Laboratory of Toxicology has continued in its efforts to increase the levels of interaction with other segments of the Drug Development Linear Array. Efforts to improve the awareness level of mutual problems in the preclinical and clinical areas continue to be fruitful. For more than two years, this Laboratory has participated in Phase I, II and III Meetings and this attendance has been rewarded with many mutually beneficial exchanges of information. These exchanges of information have been translated wherever necessary to follow-up laboratory efforts either inhouse or via the contract mechanism. Members of the Prime Contractor's Staff also have attended these clinical meetings and, in fact, have extended the level of interaction by site-visiting many of the Phase I clinicians in order to see first-hand the actual work situation for these individuals. Some members of the Laboratory of Toxicology have continued their collaboration with the Membrane Transport Section, OAD, ETP, DCT, in peritoneal transport studies and in the application of the dogfish shark as a model to study blood-brain-brain-barrier phenomena of antineoplastic agents and model compounds. These studies have been summarized elsewhere. In another collaborative effort involving the Office of the Director, DCT and the Medical Oncology, We have begun studies on the potential use of intraperitoneal drug administration in attenuating the intra-abdominal complications associated with ovarian cancer. The current phase of this study has progressed from a "library mode" to an early laboratory phase. Studies involving the use of rats and monkeys were initiated to study the transport properties of peritoneal membranes. The major goal of this work is to attempt to achieve high intra-tumoral levels of antineoplastic agents while minimizing systemic absorption and toxicity. The Laboratory of Toxicology has assembled people with the requisite combination of skills to approach this problem since it involves the collaboration of morphologic pathologists, pharmacologis surgeons and toxicologists. Personnel from the Medical Oncology Group have continued to provide advice as to the clinical relevancy of the proposed research. If any likely treatment-regimens are developed in

the animal studies, the Medical Oncology Group will implement them in patients.

E. Collaborative Efforts With Other NIH Units: Collaborative efforts of the Laboratory of Toxicology with a number of other NIH units have continued. The major basic efforts in these programs have been in the fields of pharmacology and toxicology. These studies stem from realizing the important obligations those of us who work with drug xenobiotics owe to our less fortunate colleagues working with other xenobiotics. The over-all predictive value in extrapolating effects from animals to man is poor for most xenobiotics. But this is primarily due to the inadequacies of the human toxicologic data and secondarily due to the nature of animal test procedures. Precise knowledge of dose and schedule are virtually impossible to obtain in the case of environmental agents such as pesticides. Because those of us working with drugs can more precisely analyze the relationships we have uncovered between animal and human toxicity, and because we know the dose and schedule, we have an extra obligation to pass on our findings to our colleagues working with non-drug xenobiotics. The basis for this statement is that since these foreign compounds apparently obey the same general principles in regard to interactions with organisms, then the predictiveness of our work when extrapolated from animals to man, becomes a cornerstone for other investigators. As was reported in past annual reports, such studies often lead to a convergence of environmental and cancer research areas. For example, collaborative studies with the Biomedical Engineering and Instrumentation Branch, DRS, have centered around studies of the physiologic disposition of anticancer agents containing platinum, e.g., cis-diamminedichloro platinum. Several federal agencies have been anticipating the potential problem of platinum waste in the biosphere since this heavy metal is used in catalytic converters in motor vehicles. Since it is estimated that such catalysis would not last more than five years, the work this research team is doing to solve an antineoplastic drug problem, also is important in establishing the background data on the disposition of platinum compounds in animals. Furthermore, by using highly sensitive non-isotopic methods, they have made available to this biomedical community, methods applicable to humans. Once the disposition of this agent in animals is understood, it will be rather facile to extrapolate such studies to what might happen if platinum wastes are absorbed by humans. Other work done in collaboration with NIEHS has centered around studying marine pharmacology at several levels. While the bases for these studies have been spelled out elsewhere, it should be mentioned here that this work has widespread relevance, since the oceans are the "ultimate sink" for all xenobiotics. Hence those who are best qualified to do so - pathologists, pharmacologists and toxicologists - should characterize the effects of xenobiotics on marine species and the effects of such species on xenobiotics. This baseline information is of importance to all mankind since it will raise the awareness level that a resource like the ocean cannot be polluted indefinitely by drugs and other xenobiotics.

III. Contract Program

A. The Prime Contract: We have now completed two years of operation under the Prime Contract mechanism. The technical aspects of this program are working out exceedingly well. During the first contract year, the Prime awarded two subcontracts and in the second year awarded three additional subcontracts. The major goal of the Prime Contract is to continue the protocol toxicity studies in large animals and it has served this goal very well by completing the first assigned protocol tasks within eight to nine months. Not only has the Prime provided the Division with a mechanism for the rapid completion of such studies an average of 40% faster, it has done so with some cost savings, e.g., about 20%. During the second contract year again only about one-half the projected number of protocol drugs were available to the Prime, but for the present contract year it would appear that a large increase in their workload will result from new assignments particularly in the areas of radiosensitizers and drug-radiation modalities. The major impact of the Prime has been that it has made available more research time for the internal staff, so that they can pioneer methodology which when adequately worked out, can then be taken over by the Prime. For example, a rat model to assess the relative nephrotoxicity of a series of platinum alalogues was developed this year. This type of a developmental role is imperative in a rapidly changing field such as is modern toxicology.

B. Present Contract Programs: Most of the contracts supported by the Laboratory of Toxicology for protocol toxicity studies were terminated as of March 1974 and to date, three of this original group have been brought aboard under the Prime Contract mechanism. The Laboratory of Toxicology also supervises four other contracts. Two of these are in the area of drug metabolism wherein these groups (University of Oregon and Stanford Research Institute) are conducting studies primarily on the metabolism and other apsects of disposition of antineoplastic agents. Colorado State University is under contract to study the cardiotoxicity of adriamycin and this contract has been very fruitful in aiding the Laboratory in developing the rabbit model as mentioned earlier in this report. The fourth contract is with Dow Chemical Company and is involved with reproductive and teratogenic toxicity of antineoplastic agents. This resource was developed in answer to FDA's request to provide such information for antineoplastic agents at the NDA phase. This has been a very productive contract in that in the past four years, they have completed full studies for 10 agents and this information has already been used or is about to be used to support several NDA applications to the FDA.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03518-08 LT

PERIOD COVERED
July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)
Pharmacologic Interruption of the Metabolism of the Dicarboxylic Amines and their Amides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Cooney	Senior Surgeon	LT, ETP, DCT, NCI
Others:	H. N. Jayaram	Staff Fellow	LT, ETP, DCT, NCI
	Benjamin Taylor, Jr.	Staff Fellow	LT, ETP, DCT, NCI
	S. Anandaraj	Visiting Fellow	LT, ETP, DCT, NCI
	Harry A. Milman	Research Assistant	LT, ETP, DCT, NCI

COOPERATING UNITS (if any)

Georgetown University; Arthur D. Little, Inc.; Frederick Cancer Research Center; University of Delaware; Sloan Kettering Institute

LAB/BRANCH
Laboratory of Toxicology

SECTION
Biochemistry

INSTITUTE AND LOCATION
NCI, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	4	1

SUMMARY OF WORK (200 words or less - underline keywords)

Use of the oncolytic enzyme, L-asparaginase, demonstrated that deprivation of a non-essential amino acid could have lethal repercussions on tumor cells. This Laboratory is exploring alternate means of achieving such deprivations by inhibiting L-asparagine synthetase, L-asparaginyl-tRNA synthetase, and stimulating endogenous L-asparaginase activities. Orthodox drugs and inter-mediary metabolites are among the agents being examined in a systematic way for such actions. In addition, inasmuch as L-glutamine is the ordinary source of the nitrogen of L-asparagine, we are exploring a select number of powerful L-glutamine antagonists for their perturbation of L-asparagine metabolism. Lastly, a small number of new oncolytic enzymes are being subjected to therapeutic and toxicologic testing.

Project Description:

Objectives:

To characterize the biochemical basis for the toxicity of select oncolytic drugs, and, where possible to devise and develop antidotal measures.

Methods Employed:

Standard enzymatic, biochemical and analytical techniques have been utilized.

Major Findings:

I. Continuing Studies of L-Asparagine Metabolism

i) Background: The biosynthesis of L-asparagine proceeds readily in experimental tumors resistant to L-asparaginase and is attributable to an increase in their content of L-asparagine synthetase. Inhibition of this enzyme has been suggested as a means of conferring sensitivity to L-asparaginase on resistant tumors by nullifying their mechanism of resistance. One of the laboratory's continuing goals is a search for effective inhibitors of this enzyme.

Mammalian pancreas contains L-asparagine synthetase at the highest specific activity of any tissue in the body. In the mouse, this activity is comparable to that found in the L5178Y/AR tumor. By contrast, it is mammalian liver which contains the hydrolyzing enzyme (L-asparagine amidohydrolyase, L-asparaginase) at the highest specific activity of any tissue; liver also has been shown to regulate the levels of L-asparagine in blood. These enzymes can be viewed as a tandem pair, operating antagonistically in the control of L-asparagine metabolism.

ii) A Search for Endogenous Regulators of L-Asparagine Synthetase from Mouse Pancreas and Murine Tumors: To date, over 500 drugs and chemically-reactive molecules have been examined as potential inhibitors of L-asparagine synthetase. We reasoned that another potential type of control of this important enzyme would involve its interaction with intermediary metabolites. Therefore the ability of 300 naturally-occurring compounds to influence the biosynthesis of L-asparagine has been examined *in vitro* using the enzyme from L5178Y/AR, exposed to a modulator-concentration of 10 mM. Only 15 inhibitors were uncovered; these were mainly trace metal salts such as the chlorides of calcium and zinc. However, lithium hydroxypyruvic acid and guanidinosuccinic acid both emerged as strongly inhibitory. In every case so far examined, an agent inhibitory to the tumoral enzyme has been comparably inhibitory to its pancreatic counterpart. It is concluded that mammalian L-asparagine synthetases are relatively refractory to metabolic controls. To fortify this conclusion, a more comprehensive catalogue of intermediary metabolites will be examined.

iii) Miscellaneous Inhibitors of L-Asparagine Synthetase: Since our

previous report, we have tested an additional 100 miscellaneous chemicals and drugs, in vitro, against L-asparagine synthetase of Leukemia 5178Y/AR. Of these, only one (homoserine-AMP, a transition state analogue) produced greater than 50% inhibition of the enzyme at a concentration of 0.05 mM. Preliminary data indicate that the drug-magnesium complex is the active species. When tested as an inhibitor of L-asparagine synthetase of mouse pancreas (specific activity approximately 0.04 I.U./mg) the agent was found to be uncompetitive versus L-glutamine, ammonia and L-aspartic acid. Studies with ATP.Mg++ as the variable substrate are in progress. Interestingly, S-adenosyl methionine and S-adenosyl homocysteine were without activity up to 1.0 mM concentrations. However, at 3 mM, S-adenosyl methionine totally inhibited the synthesis of L-asparagine, whereas even a concentration of 5 mM of S-adenosyl homocysteine was without effect. The role of magnesium chelation in these effects is being studied further.

At 10 mM, L-cysteine sulfonic acid, an analogue of L-aspartic acid, inhibited tumoral L-asparagine synthetase by 65%. Twelve other analogues of L-cysteine were tested as inhibitors, but none produced greater than 37% inhibition at a drug concentration of 10 mM. It is noteworthy that S-2,3-dicarboxyaziridine, an internal aziridine analogue of L-aspartic acid isolated and provided by Umezawa this year, was wholly inactive as an inhibitor of L-asparagine synthetase.

iv) The Purification and Biochemistry of Mouse Pancreatic L-Asparagine Synthetase: Pilot purification techniques were enumerated in previous reports. A final scheme for the isolation of L-asparagine synthetase mouse pancreas is presented below:

Technique	Specific Activity (nmole/mg/hour)	Percent Recovery
1. Crude homogenate	7.4	100.0
2. 105,000 g ultracentrifugation	31.9	100.0
3. DEAE-Sephadex, A-25, pH 6.0	1097.3	80.0
4. DEAE-Sephadex, A-25, pH 7.8	3803.1	40.0
5. Sucrose Gradient ultra-centrifugation	5896.7	8.0

A tris-formate (0.01 M) gradient is used in the ion-exchange procedures (steps 3 and 4, above); 0.5 mM disodium-EDTA and 1 mM dithiothreitol are used to stabilize the enzyme in steps 1 through 4. Step 5 enzyme must contain 10 mM of this thiol inasmuch as lower concentrations are insufficient to prevent oxidation of the so-called L-glutamine site. Enzyme from steps 1,2 and 3 may be stored at -87° with no appreciable loss of activity for at least one month. When step 4 enzyme is stored at -87°, however, the L-glutamine site is completely destroyed, presumably due to oxidation. Dithiothreitol at 10 mM prevents this destruction. In like manner, the sucrose gradient ultracentrifugation step must be conducted in the presence of 10 mM dithiothreitol to prevent oxidation at the L-

glutamine site. Step 4 enzyme has been used to characterize patterns of inhibition produced by various prototypical inhibitors of L-asparagine biosynthesis. Aminomalonic acid proved to be competitive with L-aspartic acid, but uncompetitive with L-glutamine, ammonium chloride and ATP-$MgCl_2$ When the ratio of ATP:$MgCl_2$ was 1:1 (10 mM:10 mM). DONV (5-diazo-4-L-norvaline however, was competitive with both amide donors (L-glutamine and NH_4Cl) but non-competitive with L-aspartic acid and uncompetitive with ATP:$MgCl_2$) when the ratio of ATP:$MgCl_2$ was 1:1 (10 mM:10 mM).

Step 4 enzyme exhibited Michaelis Constants, for L-glutamine, L-aspartic acid, ATP and ammonium chloride of 0.0004 M, 0.0001 M, 0.0066 M and 0.003 M, respectively. Furthermore, the V_{max} of this enzyme was markedly affected by the ATP:$MgCl_2$ ratio: a 1:5 (ATP:$MgCl_2$) ratio was found to produce maximum velocity. This enzyme also was found to be inhibited by its products. L-asparagine was the most powerful product inhibitor, producing 75% inhibition of the enzyme at 0.5 mM. A 37% inhibition of the enzyme was produced by an AMP concentration of 1 mM and 48% at 7.5 mM. L-Glutamic acid was the least active product inhibitor producing only 22% inhibition at 20 mM.

v) Use of L-Asparagine Synthetase as a Marker for Human Neoplasia: In a previous report we demonstrated that L-asparagine synthetase leaked into the circulation during the growth of some, but not all, murine leukemias. As a logical extension of these studies we have now expanded this survey to a small number of serum samples from patients with neoplasms; samples were provided by the serum bank of the Mayo Clinic. In the one hundred sera sent, no evidence for the presence of L-asparagine synthetase could be uncovered despite the fact that ultrasensitive methodology was used and that a counteragent, (EGTA) to the principal endogenous inhibitor present in plasma (calcium ions), was utilized in the measurements.

vi) A Search for Endogenous Regulators of L-Asparaginase from Mouse Liver: Many enzymes are subject to modulation by their products. We reasoned that hepatic L-asparaginase would be no exception to this generalization. If the modulation were to be positive (i.e., stimulatory) and powerful, we reasoned that it ought to be possible to perturb the homeostasis (chemostasis) of L-asparagine by administration of the modulator. This technique might be a means of starving suitable tumors of a metabolite which is quasi-essential for them. Towards, this end, a search is being made for regulators of hepatic L-asparaginase among 300 intermediary metabolites, ions and other naturally-occurring materials. Testicular L-asparaginase is being studies also for purpose of comparison.

To date the in vitro analyses are complete with 300 putative modulators at a concentration of 10 mM. Under these 'pharmacologic' conditions, many powerful inhibitors and stimulators were observed. Fumarate, for example, inhibits hepatic L-asparaginase by 97%, whereas citrate activates the enzyme three-fold. Inasmuch as both of these intermediary metabolites are ultimately derived from L-aspartic acid, it is obvious that a feed-

back system of control exists in the case of hepatic L-asparaginase. Further studies in vitro as well as in vivo - aimed at characterizing this control are planned using concentrations more likely to prevail in vivo.

vi) A Study of Changes in the Activity of Testicular L-Asparaginase with Growth Development in the Mouse: In mammalian testes, L-asparaginase exists at a concentration second only to liver. Nevertheless, the testicular enzyme has so far not been characterized. As a first step toward such characterization, we have measured the specific activity of L-asparaginase in the testes of pre-pubescent, pubescent, adult and senescent mice. The finding that the enzyme increases in concentration at the time of onset of spermatogenesis suggests that this L-asparaginase is under humoral control in vivo. A comparison also was made of the apparent affinity of testicular L-asparaginase for L-asparagine: the Michaelis Constant was 0.003 M; this should be contrasted with the Km of hepatic L-asparaginase (0.02 M). Apparently the testicular enzyme has a notably greater affinity for its substrate - although definitive statements on this point must await purification of the enzymes.

As mentioned earlier, the influence of a large panel of naturally occurring compounds on both hepatic and testicular L-asparaginase is being carried out in order to probe the regulatory mechanisms operative in the case of these hydrolases. Although the study with testicular enzyme is only midway, it appears that this L-asparaginase, unlike its hepatic counterpart is rather refractory to regulation by intermediary metabolites.

viii) Initiation of the Purification of L-Asparaginyl tRNA Synthetase from Tumor and Pancreas: L-Asparaginase is believed to achieve its oncolytic action by deleting an amino acid required for tumor-protein synthesis. As other workers have pointed out, it ought to be possible to achieve the same end by efficacious inhibition of tRNA synthetase. Preparatory to the initiation of a search for such inhibitors, we have carried out assays for this enzyme in extracts of Leukemia 5178Y/AR as well as in normal mouse pancreas, inasmuch as these tissues represent important sites of L-asparagine metabolism. The enzyme was readily demonstrated in the tumor but absent from pancreas. However, after the 105,000 supernatant from pancreas was chromatographed on DEAE-Sephadex, G-25 (formate form), this synthetase was found in the effluent fractions eluting at approximately 0.35 M Tris-formate. Thus, it appears that the enzyme from this source is, in fact, inhibited in the crude homogenate used, and, by extension perhaps even in vivo. Further studies on the inhibition of L-asparaginyl tRNA synthetases from pancreas and tumor are projected.

II. Studies of Oncolytic Amino Acid Antibiotics

i) Biochemical, Pharmacologic Studies with Alanosine (NSC-143,647) (Propionic acid, 2-amino-3-(hydroxynitrosamino)-, DL -): The new antitumor antibiotic, Alanosine, is at present in Preclinical Toxicology; our colleagues

have demonstrated that the drug is excreted principally in the urine, but that its first carbon is detached and exhaled to an important degree (16%). Subsequently, we have begun a study of the metabolic pathways responsible for the oxidation of this amino acid. To date it has been established that DL-Alanosine is a substrate for the L- and D-amino acid oxidases, and that the drug is readily transaminated by crude and purified transaminases. Heart is the most active source of such crude preparations and GOT is the most active crystalline enzyme found capable of carrying out the reaction; α-ketoglutarate is the preferred partner. On these grounds it is suggested that alanosine is metabolized to the keto acid (α-keto alanosine) which is then decarboxylated either by H_2O_2 (in the case of the oxidases) or by further intermediary metabolism, in the case of the transaminases. Indeed preliminary results suggest strongly that α-keto alanosine can enter the citric acid cycle.

The nature of the principal urinary metabolite is also being studied. This acidic moiety has lost a free α-amino function but appears to retain both β-nitrogen substituents. It has been purified by chromatography on DEAE-sephadex and high-voltage paper electrophoresis and is being subjected to mass-spectrometry.

ii) Interaction of Alanosine with Enzymes Acting on the Dicarboxylic Amino Acids and their Amides: The recurrent theme in the literature on alanosine is that this antitumor agent is a functional analogue or antagonist of L-aspartic acid. To characterize this antagonism more fully a comprehensive analysis is being made on the interaction of the antibiotic with the enzymes acting on or degrading the dicarboxylic acids and their amides. Adenylosuccinate synthetase, argininosuccinate synthetase, L-aspartate transcarbamylase, aminoimidazole-N-succinocarboxamide ribonucleotide synthetase, L-aspartate N-acetylase, L-aspartyl-tRNA synthetase, L-aspartase, L-aspartate β-decarboxylase, L-aspartyl kinase, L-glutamate oxaloacetate transaminase, L-glutamate pyruvate transaminase, γ-L-glutamyl L-cystein synthetase, γ-L-glutamyl transpeptidase, L-glutamyl-tRNA synthetase, L-glutamine synthetase, L-glutamate synthetase, L-asparaginase, L-asparagine transaminase, L-asparaginyl tRNA synthetase, L-glutaminases, L-glutaminyl-tRNA synthetase, L-asparagine synthetase, fructose-6-phosphate amidotransferase, formyl glycinamide ribonucleotide amidotransferase, CTP synthetase, GTP synthetase and PRPP-amidotransferase, comprise the enzymes whose interactions with alanosine have been examined thus far.

Most workers have attributed the oncolytic action of this antibiotic to its inhibition of adenylosuccinate synthetase. Using a crude enzyme-preparation from murine muscle, we were unable to demonstrate any significant inhibition by alanosine of this important enzyme. However, high concentrations of alanosine do inhibit L-aspartyl-tRNA synthetase, glutamate oxaloacetic acid transaminase and L-glutamine synthetase from brain. The contributions of these modest inhibitors to the mode of action of alanosine remain to be clarified. The remaining enzymes acting on the dicarboxylic amino acids were resistant to inhibition by L-alanosine.

In addition to the above mentioned studies, some in vivo studies on the biochemical effects of alanosine were carried out. Of significance is the pronounced L-aspartic-aciduria and L-glutamic-aciduria that the drug induces in the mouse.

III. Alkylation of Nucleic Acids by Streptozotocin (Glucopyranose-2-deoxy-2-(3-methyl-nitrosoureido-D-) NSC-85,998)

i) Correlation of Alkylation and Resistance: In order to determine whether resistance to Streptozotocin was mediated by resistance of target nucleic acids to alkylation by the drug, we have compared the conversion of guanyl to 7-methyl guanyl residues in the DNA of tumors sensitive and resistant to the drug. In the sensitive variants, 1 - 24 hours after the injection of 20 μCi of methyl [^{14}C]-Streptozotocin, less than 75% of the radioactivity was shown by paper chromatography to be 7-methyl guanyl residues. A comparable percentage of alkylation was seen in the resistant variant although the absolute level of radioactivity associated with DNA isolated in this case was only 50% of that seen in the DNA of the sensitive variant. This may be indicative of a difference in up-take of the drug.

Because nicotinamide nullifies the diabetic but not the oncolytic action of Streptozotocin, we have also explored the possibility that the vitamin might be shielding nucleic acids from alkylation. In vitro, at pH's varying from 4 to 7, the methylation of guanyl residues by Streptozotocin was not significantly altered by nicotinamide concentrations as high as 0.1 M.

IV. Cytotoxicity of Shark Plasma

i) Definition of the Cytotoxic Effect: In the course of an investigation into the ability of the serum and plasma of several diverse species to alter the growth of murine lymphoblasts in culture, we observed that the plasma of the dogfish shark (Squalus acanthias) is cytotoxic to a subline of Leukemia 5178Y (cells rendered resistant to L-asparaginase) and to L1210 cells in vitro. Morphological examination of cells cultured for 48 hours in shark plasma revealed a striking megalocytosis of the treated cells. Electron-particle-sizing-analysis of the cells grown in fetal calf serum and of cells cultured in shark plasma indicated a three-fold increase in the mean cell volume of lymphoblasts cultured in shark plasma. Light and electron microscopic studies of the cells revealed a decrease in nuclear heterochromatin in treated cells. Since shark plasma either failed to support or interrupted cell division, attempts were made to localize the phase of the cell cycle at which this supplement was operating, using cytofluorimetric analysis of the DNA distribution of cells. Cell cycle progression appeared to be arrested primarily in the G_1 phase. Measurements of the synthetic rate of DNA, RNA and protein in L5178Y/AR cells, at early time periods (up to 4 hours) indicate that the synthesis of all three macromolecules proceeds at almost the same rate in cells treated with fetal calf serum or shark-plasma. Dialysis of shark plasma did not alter its cytotoxic effect. Reinoculation in vivo of the lymphoblasts treated

with shark plasma in vitro for 24 hours indicates that the treatment with shark plasma did not result in the loss of viability of the residual tumor cells. Acrylamide gel electrophoretic analysis of the shark plasma in stacked gels (3.5, 4.75 7.0 and 12.0%) indicated three major families of protein bands, one each in the 3.5, 4.75 and 7.0% gel. One of the major bands (in the 7.0% region) migrated with the same mobility as mammalian pre-albumin. Attempts are under way to determine whether any of these proteins is responsible for the cytotoxic effect reported here.

V. Studies with New Antitumor Enzymes

i) L-methioninase (NSC): This enzyme is active against Walker carcinosarcoma. Preparatory to purification here by a new scheme which entails the direct loading of the lysate on large columns of DEAE-Sephadex, a kilogram of the bacterial cells is being grown at the Frederick Cancer Research Center. It is anticipated that the partially purified enzyme will be subjected to screening versus P388 by June 1976.

ii) L-Phenylalanine Ammonia Lyase A small supply of this oncolytic enzyme was purchased from P. L. Laboratories and subjected to antitumor testing in the Laboratory of Toxicology. On day zero, sixteen male BDF_1 mice were given 1×10^5 cells of L5178Y, ip. The next day, six of the tumored mice received 400 I.U./kg, ip, of L-phenylalanine ammonia lyase (2 I.U./mg protein). Controls received saline. The enzyme-therapy was repeated for three more days. The median survival for the treated group was 15 days (T/C=111%) and for the saline controls was 13 days. There were no survivors. It should be pointed that acrylamide gel electrophoresis of this preparation showed it to be heterogenous. One of the impurities was identified here as urease. This contaminant will give rise to a large ammonia load in vivo. Pursuant to the foregoing negative results, and inasmuch as the clearance of PAL is subject to very prompt immune-acceleration, (as early as 3 days after the first exposure the $t_{1/2}$ can fall from 8 to 2 hours), steps are being taken to encapsulate the enzyme in liposomes. These artificual cells will be used to treat mice bearing P388 in a comparison study using equal unitage of the free enzyme.

iii) L-Asparaginase from Vibrio succinogenes: All the bacterial L-asparaginases tested in the clinic so far are very active L-glutamine amidohydrolases as well. The enzyme from Vibrio succinogenes was reported to be an exception to this rule. Procurement of a small sample of the purified enzyme permitted us to test this point with sensitive radiometric methodology. L-Glutamine was in fact hydrolyzed by the enzyme, but at a rate less than 0.1% of that exhibited with L-asparagine as substrate. Inasmuch as the L-glutaminase activity of E. coli and Erwinia carotovora L-asparaginases have been implicated in their toxicity the study of this enzyme will be of toxicologic interest.

VI. New Analytical Methodology

i) A Unitary System for the Measurement of Enzymes Utilizing L-Aspartic Acid as Co-substrate: Confronted with the problem of measuring large numbers of enzymes utilizing L-aspartic acid, we were forced to develop a unitary method of assay. Because there are theoretical disadvantages to the assessment of substrate-disappearance (as opposed to product-appearance) we designed a broadly-applicable system which has as its principal the acceptor-dependent conversion of L-[4-^{14}C]aspartic acid to a form not subject to transamination by L-glutamic acid oxaloacetic acid transaminase (GOT). This latter enzyme has strict substrate specificites and will not accept the modified amino acid. In practice radiolabelled L-aspartic acid is incubated with its co-substrate, (for example L-[^{14}C]aspartic acid is incubated with carbamyl phosphate and a crude preparation of L-aspartate transcarbamylase.) After a suitable incubation, the reaction mixtures are heated briefly at 95° to terminate the reaction, and residual or unreacted L-aspartic acid is dissipated with GOT in the presence of α-ketoglutarate and zinc ions, as described in a previous annual report. After 12 hours at 25°, the residual radioactivity is counted in a scintillation spectrometer. When suitable controls are included, it is possible to document that the residual radioactivity is co-substrate dependent and to correct appropriately for blank radioactivity. The system works well with most of the L-aspartic-acid-utilizing-enzymes treated in the section on alanosine (II, supra); however, L-aspartyl-tRNA synthetase and aminoimidazole succinocarboxamide ribonucleotide synthetase cannot be estimated by the technique because of the comparatively feeble activities.

ii) Ion Exchange Chromatographic Separation of Methylated Bases: In the present year, chromatographic methodology for the isolation and quantitation of eight of the methylated derivatives of nucleic acids which have been most often mentioned as alkylation products has been substantially improved. The procedure utilizes the long column of the amino acid analyzer and two lithium citrate buffers (0.15 and 0.30 M, respectively); the first at pH 5.97 and the second at pH 8.0. Column temperature is maintained throughout at 57° and the buffer is changed at 120 minutes. Monitoring of the effluent is achieved using a 2-cell U.V. detector at 260 nm and 280 nm respectively, a fluorescence-monitor especially adapted to take advantage of the characteristics of O-6 guanyl residues (excitation 304 nm and emission 370 nm) and a radiometric cell in series. Under these conditions, guanine and its other derivatives give minor fluorescent peaks while as little as one nanomole of the O-6 species can be measured with accuracy.

Significance to Biomedical Research and the Program of the Institute:

Ideally, the Biochemistry unit uncovers the biochemical mechanisms underlying the toxicity of new oncolytic drugs and suggests rational antidotes for this toxicity. The stress is on enzymology, inasmuch as most drugs operate by interacting with enzymes. Practically speaking,

this unit cooperates with the Laboratory of Toxicology as a whole, and with the Division of Cancer Treatment in ensuring that new chemotherapeutic modalities can be used safely in man.

Proposed Course:

It is proposed to pursue our evaluation of L-asparagine synthetase as a marker for resistance, as an index of neoplasia, and as an intrinsically important biosynthetic enzyme.

Publications

1. Cooney, D. A., Milman, H. A., Jayaram, H. N. and Homan, E. R.: Inhibition of L-asparagine Synthetase by Mucochloric and Mucobromic Acids. Enzyme (in press).
2. Cooney, D. A., Driscoll, J. S., Milman, H. A., Jayaram, H. N. and Davis, R. D.: Inhibitors of L-asparagine Synthetase, In vitro. Cancer Treat. Rep. (in press).
3. Cooney, D. A., Milman, H. A. and Taylor, Jr., B. A Radiometric Method for the Measurement of L-asparaginase at Concentrations Below 1 x 10^{-5} I.U./ml. Technique and Application. Biochem. Med. (in press).
4. Cooney, D. A., King, V. D., Cable, R. G., Taylor, Jr., B. and Wodinsky, I. L-asparagine Synthetase in Serum as a Marker for Neoplasia. Cancer Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06110-06 LT

PERIOD COVERED

July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Pathology of Drug-Induced Alterations in Animals and Man.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David M. Young	Comparative Pathologist	LT, ETP, DCT, NCI
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	Arnold W. Pratt	Director	DCRT, NIH

COOPERATING UNITS (if any)

Division of Computer Research and Technology

LAB/BRANCH

Laboratory of Toxicology

SECTION

Comparative Pathology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	3.5	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

The major objective of this project is to define and characterize animal models for (a) pathogenesis studies of anticancer agent-induced lesions, and (b) comparative investigations of neoplastic diseases. Specific studies are designed to:

1) Characterize the cardiomyopathic effects of Adriamycin in animals;
2) Characterize the transplantable VX-2 carcinoma in rabbits as a model for osseous-mediated hypercalcemia of malignancy;
3) Evaluate morphological and biochemical alterations of skeletal muscle induced by Adriamycin;
4) Define an animal model for chondro-osseous neoplasia;
5) Evaluate the rat as a model for study of nephrotoxic effects of anticancer agents;
6) Characterize the biological behavior of various animal and human neoplastic processes transplanted to nude (nu/nu) mice;
7) To systematically evaluate the clinical and autopsy records for evidence of adverse effects of antineoplastic agents in cancer patients on a computerized basis and correlate these findings with those recorded in test animals.

Project Description:

Objectives:

1. To define the clinicopathologic effects of adriamycin (ADR) in rabbits, mice and rats and characterize an animal model for:
   a. analogue testing
   b. antidotal testing
   c. antitumor and cardiotoxicity testing

2. To characterize the physicochemical and morphologic alterations in chondro-osseous tissues induced by long-term ADR administration.

3. To characterize the transplantable VX-2 carcinoma in rabbits as a model for osseous-mediated hypercalcemia of malignancy.

4. To evaluate morphologic and biochemical alterations of skeletal muscle in rabbits with ADR-induced cardiomyopathy.

5. To define an animal model for experimental chondro-osseous neoplasia for:
   a. investigation of biologic behavior and pathogenesis
   b. testing antineoplastic agents for activity against chondro- and osteosarcomas.

6. To evaluate the adverse effects of ADR on calcium homeostasis and to correlate these findings with the known calcium accumulation in affected myocardia.

7. To evaluate the rat as a model for determining nephrotoxic effects of platinum containing antineoplastic agents.

8. To determine the role of lysozyme in chondro-osteogenesis under physiologic and pathologic conditions.

9. To characterize the hematopoietic and chondrogenic lesions in Alaskan Malamutes with hereditary chondrodysplasia as a model for dwarfism in humans.

10. To characterize clinicopathologically the effects of transplantation of various neoplasms to athymic nude (nu/nu) mice.

11. To systematically evaluate the clinical and autopsy records for evidence of adverse effects of antineoplastic agents in cancer patients on a computerized basis and correlate these findings with those recorded in test animals.

12. To provide advice, consultation and support in the area of pathologic evaluation for senior investigators within the DCT.

Methods Employed:

1. Standard procedures for pathologic evaluation of tissues and body fluids were employed.

Major Findings:

1. The Clinicopathologic Effects of ADR in Rabbits, Rats, and Mice. Recent reports of the toxic effects of ADR in animals and humans indicate that the tissues of the following eight organ systems are adversely affected: hematopoietic, lymphoreticular, digestive, reproductive, cardiovascular, urinary, skeletal and integumentary. Myelosuppression, digestive disturbances, stomatitis and alopecia are the most frequently occurring adverse effects in patients receiving ADR. It has been well accepted that the cardiotoxicity of ADR is the major total dose limiting toxic manifestation in patients. A number of animal models have been characterized to various degrees as indicators of ADR-induced cardiotoxicity. An animal model would be valuable in testing analogues of ADR for cardiotoxicity and for testing antidotal procedures. To date, only the rabbit and monkey have been reported to develop a dose-related cardiomyopathy with congestive heart failure. Smaller laboratory animals (i.e. mouse and rat) offer distinct advantages as models with respect to size, temperament, handling, care and housing required for testing large numbers of drugs. Therefore, the current investigations are designed to determine if ADR will induce a significant cardiotoxic syndrome in the rat and/or mouse. Preliminary evidence suggests that the mouse develops a cardiomyopathy after single as well as multiple doses of ADR. The morphologic changes appear to be similar, but to a lesser degree in severity, to those described in the rabbit and humans. The structural alterations correlate well with the findings of decreased myocardial DNA production and increased calcium accumulation in heart muscle. These findings are being compared with those reported in the rabbit and with those under current investigation in the rat.

2. Physicochemical and Morphologic Alterations in Chondro-Osseous Tissues Induced by ADR. Long-term, low dose administration of ADR to young growing rabbits resulted in morphologic changes which were most pronounced at epiphyseal and metaphyseal areas of long bones. Results of in vitro studies indicate that ADR binds readily to nondemineralized, fresh cortical bone powder.

The findings in ADR treated rabbits of decreased bone density, histopathologic alterations, and a paucity of osteogenic cells in ADR treated rabbits are interpreted as retardation of bone maturation. Further investigations are in progress to better define the interaction of ADR and chondro-osseous tissues.

3. Clinicopathologic Evaluation of the Transplantable VX_2 Carcinoma in the Rabbit. Recent findings indicate that various malignancies have a distinct affinity to alter skeletal morphology and biochemistry with or without osseous metastases. The pathogenesis of these cellular and humoral influences of neoplasia on skeletal homeostasis is poorly understood. The present study was designed to characterize the biological behavior of the VX-2 carcinoma in rabbits and to evaluate its usefulness as a model to

study the pathogenesis of osseous mediated hyperclacemia of malignancy. A total of 88 experimental and 25 control NZW rabbits were used. Injections of VX-2 carcinoma cells in biceps femoris muscles resulted in rapid, progressive neoplastic growth with development of a significant hypercalcemia at 14-21 days No metastases to skeletal tissues were evident. Roentgenographic and histopathologic evaluations of femora revealed varying degrees of periosteal new bone growth, cortical osteolysis, endosteal new bone growth and in a few cases, pathologic fractures. Bone lesions were not evident at sites distant from neoplastic growth.

Mineral analyses of VX-2 carcinoma tissues and kidneys from VX-2 carcinoma rabbits revealed concentrations of calcium 83 and 3 fold greater than control tissues, respectively. These findings correlated well with histochemical evidence of excessive amounts of calcium in these tissues. Ultrastructurally VX-2 carcinoma cells were characterized by pleomorphic nuclei, irregular interdigitating cytoplasmic borders with microvillar projections, abundant cytoplasmic organelles and absence of membrane bound secretory granules. Preliminary biochemical studies indicate that VX-2 carcinoma tissues contain prostaglandins.

Physiocochemical analyses of selected long bones from VX-2 rabbits revealed significantly increased wet weights and decreased densities. These results correlated well with roentgenographic and histopathologic findings of increased quantities of woven bone compared to more mature lamellar bone in sections adjacent to neoplastic growth. The results of our studies indicate that (a) the VX-2 carcinoma in rabbits is an excellent model to study the interactions of neoplasia and skeletal homeostasis, especially osseous - mediated hypercalcemia of malignancy (b) VX-2 carcinoma cells alter skeletal morphology and physicochemistry through an apparent dual humoral/cellular mechanism; (c) the humoral factor associated with hypercalcemia appears to be inactivated in the systemic circulation.

4. Evaluation of Morphologic and Biochemical Alterations of Skeletal Muscle from Rabbits Receiving ADR. Skeletal muscle toxicity has been detected occasionally in rabbits receiving total doses of ADR of 200-400 mg/M^2 at a dose schedule of 1.25 mg/kg I.V. twice a week. The histologic lesions are most frequently seen in diaphragmatic muscle and are similar to, but significantly less severe than, the cardiac lesions produced in the same animals. Skeletal and cardiac tissues were collected from 10 ADR treated rabbits and 10 controls and analyzed for activity of high energy phosphate compounds (G-6-P, ATP, CP). Neutralized tissue extracts were analyzed using a sequential-linked enzyme spectrophotometric assay for NADPH (TPNH). The ten treated animals had received total doses of ADR ranging from 205-385 mg/M^2 (aver. 292) and seven had clinicopathologic evidence of cardiomyopathy. There were no significant differences in the activities of the high energy phosphates of cardiac muscle from treated and controls. Significant decreases were observed in skeletal muscles (diapharagm, masseter, quadriceps femoris) from treated compared to control rabbits. These preliminary findings indicate that ADR more readily alters energy utilization in skeletal compared to cardiac muscle. Perhaps the skeletal muscle biochemical changes are secondary to the toxic effects of

ADR and more directly related to debilitation, weight loss and muscle atrophy. Further studies are needed to clarify the significance of these biochemical and histopathologic findings in skeletal muscles.

5. Characterization of an Animal Model for Experimental Chondro-Osseous Neoplasia. Osteosarcoma in humans is a highly malignant neoplasm with a poor five year survival rate in spite of rigorous therapeutic regimens. It has been reported recently that osteosarcoma results in death within two years of onset in 4 out of 5 cases. No useful animal model has been reported for testing various chemotherapeutic modalities. Present investigations were undertaken to evaluate chondro-osseous neoplasms induced by the FBJ virus in mice. Neoplasms developed 5 weeks post-inoculation and were readily transplantable to weanling mice, with 4 different tumor cell lines currently being maintained. Further investigations are designed to characterize and classify histopathologically these neoplasms for testing oncolytic agents.

6. Evaluation of the Adverse Effects of ADR on Calcium Homeostasis. Previous reports from our laboratory suggest that myocardial calcium accumulation may be of etiologic importance in the pathogenesis of ADR-induced cardiotoxicity. In addition preliminary studies indicate that I.V. admininstration of ADR results in hypocalcemia in rats and rabbits. Studies were designed to determine if verapamil (VP), a cardiovascular active calcium antagonist, could reverse or alleviate the ADR-induced cardiomyopathy in rabbits. Eighteen NZW rabbits received ADR I.V. (13 mg/M^2) twice/week. Eleven of these received VP I.V. (0.5 mg/kg) 10-15 minutes preceding each ADR injection and orally in the drinking water ad libitum (300 mg/l). Four saline injected controls received VP I.V. and orally in the drinking water. ADR-VP rabbits had a 100% incidence of cardiomyopathy, a median survival time of 63 days and a mean total ADR dose of 237 mg/M^2 compared to 70%, 89 days and 320 mg/M^2 respectively for ADR rabbits. All ADR-VP rabbits had clinicopathologic evidence of congestive heart failure. Histopathologically myocardial lesions were more extensive and severe than observed in rabbits receiving only ADR. These findings indicate that the cardiovascular action of VP accentuates the cardiotoxic effects of ADR. Perhaps VP and ADR have synergistic effects on myocardial calcium metabolism. These results suggest that caution should be employed when using cardioactive and/or calcium altering drugs in conjuction with ADR.

7. Evaluation of the Rat as a Model for Testing the Nephrotoxicity of Platinum Containing Anti-Neoplastic Agents. Nephrotoxicity is the major dose limiting toxicity in patients receiving Cis-diamminedichloroplatinum II (NSC-119875), a new cancer chemotherapeutic drug. Experiments were designed to determine if the F344 rat would be a sensitive model for detection of nephrotoxicity of this drug and other potentially less toxic platinum containing analogues. Six agents containing platinum (NSC-119875, 250427, 239851, 180512, 224964, chloroplatinic acid) were administered parenterally to 500 male F344 rats, 6 weeks of age, at various dose levels from LD_{10}-LD_{100}. All drugs were nephrotoxic by intraperitoneal and/or intravenous routes as determined by BUN and histopathologic evaluation of kidneys. The most toxic drugs induced degenerative and

necrotizing tubular lesions in the entire cortex and outer stripe of the medulla. Lower doses of these and the less toxic drugs induced lesions only at the outer stripe. Mineralization within the tubular epithelia and lumina was observed histologically as terminal events. Weekly IP injections induced uremia and cystic changes at the outer stripe. These experiments revealed tha the male F344 rat is a sensitive strain for the detection and study of the nephrotoxicity of the platinum drugs.

8. Role of Lysozyme in Chondro-Osteogenesis under Physiologic and Pathologic Conditions. Lysozyme is a small, cationic, ubiquitous enzyme that has been reported to be elevated significantly in the urine and serum of patients with monocytic and myelomonocytic leukemia. In addition, determination of serum lysozyme levels has been used to differentiate between two types of neutropeni Recent studies indicate that lysozyme may play a significant role in host defenses against neoplasia and may be a mediator of antitumor function of macr phages. Further studies are needed to determine the physiologic role and antitumor activity of lysozyme. Recently, a genetic disorder consisting of a deficiency of lysozyme has been reported in a strain of New Zealand white rabbits. It is suggested that these mutant rabbits may be useful for experi- ments designed to delineate the biologic role of lysozyme. Lysozyme, for whic there is no known substrate in mammalian tissues, is present in a number of cells and body fluids including cartilage where it appears as a non-lysosomal enzyme. The present studies are designed to evaluate the response of lysozyme deficient rabbits to a rachitogenic diet. The findings of this study will aid in the elucidation of the role of lysozyme in chondro-osteogenesis.

9. Characterization of the Hematopoietic Chondrogenic Lesions in Alaskan Malamutes with Hereditary Chondrodysplasia. Chondrodysplasia is the most common cause of dwarfism in man and is easily distinguishable from the patho- logic dwarfism of hypopituitarism or hypothroidism. A hereditary chondrodys- plasia occurs in Alaskan Malamutes in concert with a hypochromic, macrocytic anemia. The pathogenesis of this disorder of hematopoietic and osteogenic cells remains to be elucidated. Current studies are designed to characterize the disorder in Alaskan Malamutes utilizing roentgenographic, biochemical, histochemical, morphologic and physiochemical analyses of bones and cartilage. In addition, these findings will be compared with those reported in man to determine if the skeletal disorder in Malamutes is a good model for dwarfism i man. Further studies are designed to determine (1) if cartilage lysozyme levels are altered, (2) if aging dysplastic cartilage undergoes neoplastic transformation and (3) if the genetic defect may involve a pathologic change in a common progenitor mesenchymal cell that gives rise to osteogenic and hematopoietic cells.

10. To Characterize Clinicopathologically the Effects of Transplantation of Various Neoplasms to Athymic Nude (nu/nu) Mice. Nude mice were used to invest gate the transplantability of available tumors of dog, cat and rabbit. The rabbit VX-2 carcinoma was readily transplanted and metastasized slowly to lung of nude mice. A canine lymphoma grew slowly in nude mice and did not metasta-

size while a cat myeloproliferative disease did not grow locally nor metastasize. Maintenance of nude mice was a major problem in these studies. The most important prerequisite for successfully maintaining these mice was that they originate from pathogen-free colonies. Both sources of mice that were available (Ft. Detrick and Charles River) had viral infections (mouse hepatitis or Sendai virus) which did not permit long term maintenance. Both viral infections induced a slow wasting disease (a persistent viral infection) which resulted in the death of all mice from 2-9 months after arrival from the commercial colony. However, short term studies (1-4 month) could be performed in these mice without significant adverse effects due to these intercurrent viral processes. Future studies will involve transplantation of human breast carcinomas and ovarian carcinomas to nude mice. The biological behavior of these neoplasms will be characterized clinicopathologically.

11. Evaluation of Adverse Effects of Antineoplastic Agents in Cancer Patients
A specialized system of information storage and retrieval was designed and implemented in collaboration with the Division of Computer Research and Technology. This processing system employs natural language and the systematized nomenclature of pathology (SNOP). The major objective of the current studies is to determine the qualitative and quantitative aspects of adverse effects of antineoplastic therapy in cancer patients at the time of autopsy. Currently, clinical and autopsy records on a total of 500 cancer patients have been processed via this computerized system. The percentage of patients receiving chemotherapy, surgery, or radiotherapy was 87, 42, and 50 respectively. Only 5.4% of the patients received immunotherapy. Neoplastic diseases other than the type evident upon admission were found in 30% of the patients at autopsy. Septicemia was evident at autopsy in 51.6% of the patients. A total of 12% of the patients were in remission at the time of death. The most frequently utilized antineoplastic agents were prednisolone, vincristine, cyclophosphamide, methotrexate, cytosine arabinoside and 6-mercaptopurine. Studies are in progress to correlate specific lesions with various treatment modalities. The anticancer agent-associated lesions in patients will be compared with those reported previously in preclinical toxicologic studies in dogs and monkeys.

12. Advice, Consultation and Support in the Area of Pathologic Evaluation.
Usually, in order to perform investigations of high scientific merit in the biomedical area, experimental animals are required. It is well known that spontaneous diseases develop in laboratory animals and are inherent problems that must be solved by a thorough understanding of the pathogenesis, and etiology of the condition. Spontaneously occurring diseases of laboratory animals have not been thoroughly investigated. In fact, new infectious agents are discovered each year. A cooperative study involving various investigators is necessary in order to elucidate the mechanism by which laboratory animals acquire, disseminate and are affected by these various agents. Intercurrent infectious diseases in laboratory animals is one of the major complicating factors in investigational studies. Recently, for example, athymic "nude" untreated mice in the Division of Cancer Treatment were found to have a fulminating pneumonia which may be caused by a new murine virus. Studies are in progress in collaboration with Microbiological Associates to isolate the

causative agent. Other diseases of experimental animals should be brought to the attention of veterinary pathologists.

Significance to Biomedical Research and the Program of the Institute:
The need for a better understanding of the pathogenesis of anticancer drug-induced lesions in experimental and clinical studies cannot be overemphasized. The elucidation of the mechanism of ADR-induced cardiotoxicity may lead to establishment of a therapeutic or prophylactic procedure(s) to negate the cardiac failure syndrome.

The characterization of an animal model of osteosarcoma would provide a new avenue for testing various modalities against a heretofore chemoresistant tumor type. Preliminary results suggest that the viral-induced osteosarcoma in mice may be a good model for osteosarcoma in man. The characterization of animal models of organ specific toxicity for evaluation of analogues of parent drugs is an important aspect of toxicologic studies. Investigations are in progress to define an animal model for renal toxicity. Further characterization of ADR-induced morphologic and biochemical lesions in skeletal muscles may provide for a clinically applicable test for impending cardiotoxicity. In addition, these studies will contribute to a better understanding o the basic ADR-induced cardiomyopathy. The studies of interaction of neoplasti tissues and skeletal homeostasis will aid in a better understanding of the pathogenesis of disseminated skeletal metastasis. Bone is the third most comm site for neoplastic metastases and the most frequent location of disseminated breast carcinoma metastases.

Proposed Course:
The computerized data from clinical and autopsy records from cancer patients and results from preclinical toxicologic studies in dogs and monkeys are currently being summarized in manuscript form. Future studies involving tabulation and retrieval of computerized data will be handled by the prime contractor.

Intramural research activities will be continued and expanded under the following specific objectives: (a) to characterize the myopathic effects of ADR and define methods to block this toxic manifestation; (b) to better define the interactions of neoplastic disease and bone metabolism; (c) to characteriz animal models for osteogenic sarcoma, organ specific drug-induced toxicity, and hypercalcemia of malignancy; (d) to contribute to a better understanding o the role of lysozyme in physiologic and pathologic conditions.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06122-04 LT

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Drug Interactions and Other Factors Influencing the Physiological Disposition and Toxicity of Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation Branch, DRS

LAB/BRANCH

Laboratory of Toxicology

SECTION

Drug Interactions

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.9	3.9	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

In recent years, it has become common practice, particularly in clinical oncology to simultaneously administer several drugs to patients for therapeutic purposes. A vast literature, both experimental and clinical, has shown that the presence of one drug may markedly influence the intensity, duration, and pharmacologic effects of another drug. Such drug interactions may have a variety of causes; they may result from alterations in drug absorption, in the binding of drugs to plasma proteins, which may directly or indirectly influence drug distribution, drug metabolism, or drug clearance via the bile or urine. Many drugs and other foreign chemical compounds - known collectively as xenobiotics - are inactivated by enzymes present in liver microsomes, primarily by oxidation and conjugation. On the other hand, some drugs and oncolytic agents are activated by these enzymes. Our major efforts are directed at understanding how drugs interact with each other and with the biological organism so that human drug therapy can be placed on a more rational basis.

PHS-6040
(12-75)

Project Description:

Objectives:

The interaction between a drug and tissue receptors which ultimately produces a pharmacologic or toxicologic response is the product of a large number of attenuating factors. For example, the distribution of drugs within the body depends upon a balance between relative organ perfusion, the affinity of the drug for binding sites within different organs, the extent of binding of the drug to plasma proteins, and the rate of its excretion into the urine and bile. Moreover, the metabolic clearance of many drugs from the body depends upon their relative rates of biotransformation to products which are more polar and more rapidly excreted. Thus, absorption, distribution, binding, metabolism and excretion tend to dictate the steady-state plasma drug concentration under specified conditions and thus determine the pharmacologic or toxicologic response. Since alterations in these factors are known to either diminish or intensify biologic responses, the need to assess these parameters quantitatively is of obvious therapeutic significance. Studies have been continued in the Drug Interaction Unit to investigate and appraise these parameters in a variety of animal species regarding drugs and other xenobiotics in general, and oncolytic agents in particular. A large body of evidence has linked drug metabolism to drug disposition and the duration of action of many drugs. Only recently have data begun to accumulate which emphasize the important role of drug metabolism or biotransformation in drug-induced toxicity. Recent findings have demonstrated that many relatively inactive foreign chemical compounds can be enzymatically activated *in vivo* to what might be termed *proximate toxicants*.

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

A. *Acute Toxicity of Certain Foreign Chemical Compounds in Control and Phenobarbital Pretreated Mice*. The biological half-life of many drugs and other xenobiotics is determined to a large extent by the rates at which they are metabolized and/or excreted. Accordingly, the current pharmacological literature is replete with reports relating changes in drug metabolism measured *in vitro* to alterations in drug disposition or acute pharmacologic effects of these drugs. For example, it has been amply documented that the induction of hepatic microsomal mixed function oxidase (MFO) activity by pretreatment with phenobarbital is associated with a marked increase in the whole body clearance rates, decrease in the biological half lives and the duration of the acute pharmacologic effects of barbiturates such as hexobarbital and pentobarbital. Others have shown that phenobarbital pretreatment enhances the biliary excretion of certain agents. These increases in drug metabolism and excretion that accompany enzyme induction are of importance not only in chronic

toxicity studies in animals but also in human chemotherapy. On the other hand, only a limited number of studies have appeared which have attempted to correlate changes in the rates of in vitro metabolism of drugs with possible changes in the acute toxicity of the same drugs.

The proposition that altered activity of microsomal enzymes measured in vitro may reflect altered in vivo acute toxicity has never been adequately tested for the substrates routinely used to monitor MFO activity in vitro.

Twenty-four hour LD_{50} values and slopes were determined in control and in phenobarbital pretreated male mice for several drugs routinely used to assay for in vitro activity of microsomal drug metabolizing enzymes. Phenobarbital pretreatment produced the expected increase in microsomal enzyme activity but had no effect on the LD_{50} of aniline, aminopyrine, p-nitrophenol or antipyrine. A 30% increase in the LD_{50} of ethylmorphine was produced by the phenobarbital treatment. Phenobarbital pretreatment had no effect on the slopes of the LD_{50} curves.

B. Hepatic and Extrahepatic Drug Metabolism in Several Common Laboratory Animal Species. The literature contains widely discrepant reports concerning the relative MFO activity in hepatic and extrahepatic tissues of different laboratory species. On the one hand, very low levels of enzyme activity have been reported in the lungs and kidneys of rats, guinea pigs, and mice, while on the other hand, our laboratory and others have reported enzyme activities in rabbit lung microsomes equal to or slightly higher than the activities in rabbit liver. The present investigation was initiated in order to shed further light on this apparent species-related difference.

The data demonstrated that no single animal species is universally desirable for studies of extrahepatic and hepatic drug metabolism. For example, the mouse is relatively deficient in hepatic cytochrome P-450 and NADPH cytochrome c reductase but is nearly unexcelled in its extrahepatic complement of these components. In addition, no single species can be said to be most active with respect to any single organ. The rabbit liver, for example, provides the highest activity in the UDP-glucuronyl-transferase pathway toward two different substrates but is quite poor in relative ability to demethylate or hydroxylate substrates while the rabbit pulmonary system is unexcelled in all activities. At present it is not possible to relate these findings to the human, mainly because of the nearly total void of data on extrahepatic activities in humans.

C. Drug Metabolism by Microsomes from Extrahepatic Organs of Rat and Rabbit Prepared by Calcium Aggregation. Studies of the in vivo metabolism of xenobiotics by hepatic microsomes have traditionally relied upon preparation of the microsomal fraction by differential centrifugation utilizing an ultracentrifuge. Because of the expense associated with the requisite equipment and the extensive time consumed in preparation, various non-centrifugal methods have been proposed for microsome isolation.

One of the most widely studied methods has been sedimentation of the microsomes after their aggregation in the presence of calcium ions.

Microsomes were prepared from liver, lung and kidney of rats and rabbits using a Ca^{+2} aggregation method. Microsomal protein yield from the lung of both species was higher by this method of preparation as compared with ultracentrifuged samples. Specific activities of rat and rabbit pulmonary p-chloro-N-methylaniline (CMA) demethylase, biphenyl 4-hydroxylase and rat pulmonary TPNH cytochrome c reductase also were decreased. Specific activities of rabbit hepatic TPNH cytochrome c reductase, CMA N-demethylase, UDP-glucuronyltransferase and biphenyl hydroxylase were decreased by calcium aggregation. Renal enzyme activities were unchanged by this method of preparation. These data indicate an apparent species and organ difference in microsomal enzyme response to calcium aggregation.

D. The Effects of Ascorbic Acid Deficiency and Repletion on Pulmonary, Renal, and Hepatic Drug Metabolism in the Guinea Pig. Activities of the mixed function oxidase system and two soluble-fraction enzymes were measured after 7, 14, 21, and 25 days of ascorbic acid deficiency, and at 3, 7, and 14 days of recovery. The ascorbic acid content of liver, lung, kidney, and adrenals decreased to less that 10% of controls during development of scurvy, and returned to control values by day 7 of the recovery period. Both cytochrome P-450 content and aminopyrine N-demethylation decreased to 60% of control values in liver microsomes at 21 and 25 days of deficiency, whereas there was no significant change in lungs and kidneys. By contrast, NADPH cytochrome c reductase did not change in hepatic microsomes but decreased slightly in lung and kidney. In the soluble fraction, glutathione S-aryl transferase activity was decreased in scorbutic liver, slightly decreased in kidney, and unchanged in lung. N-acetylation of p-aminobenzoic acid was increased in kidneys of deficient animals, with no change in the other two organs. Partial recovery from decreases in drug metabolism produced by scurvy occurred by day 3 and was complete by day 7 for the microsomal enzymes. However, S-aryl transferase activity was not fully recovered even at 14 days. The already high N-acetylation activity of scorbutic kidneys increased further over controls at 3 and 7 days of ascorbic acid repletion, but decreased to control values at 14 days.

E. Effects of Activators of UDP-Glucuronyltransferase Activity in Lung and Liver. A wide-variety of diverse materials have been demonstrated to activate hepatic UDP-glucuronyltransferase activity when added in vitro to enzyme incubations. In several instances, the combination of two or more activators produces additive or even synergistic effects. The precise molecular mechanisms responsible for such activation are not known but it has been established that in the presence of activators, latent enzyme activities can be unmasked and expressed, activities which in the "native" state would not be measurable.

As an extension of the project described in Section B above, we studied the effects of a number of activators on UDP-glucuronyltransferase

activities in rabbit liver and lung. In particular, we were interested in whether or not the low or undetectable activities in lung (see above) could be activated and whether the patterns of activation might be different in the two organs.

Microsomal fractions prepared from rabbit livers and lungs were employed as the enzyme sources. The following materials were used as potential activators, each in a wide range of concentrations so as to determine the optimum concentration for activation: mersalyl, digitonin, cetyl trimethyl ammonium bromide (CETAB), sodium deoxycholate (DOC), Triton X-100, sodium dodecyl sulfate (SDS), diethylnitrosamine (DEN), uridine diphosphate-N-acetyl-glucosamine (UDPNAG), and Mg^{++}. The effects of the activators were studied alone and in various combinations. Because previous reports had indicated differential effects based on the substrate employed for measuring UDP-glucuronyltransferase activity two substrates were employed, viz, p-nitrophenol and o-aminophenol. The salient findings of this project may be summarized as follows:

(1) All activators exhibited biphasic effects as a function of concentration, i.e., activation at low concentrations and inhibition at higher concentrations.

(2) Mersalyl, digitonin and Triton X-100 were the most effective activators in liver. The effects of the detergents and UDPNAG were essentially additive. DEN reduced this activation. UDPNAG alone activated less than its combination with a detergent.

(3) There were no significant differences in the effects of activators alone or in combination, with the two different substrates, p-nitrophenol or o-aminophenol.

(4) None of the activators, alone or in combination unmasked measurable UDP-glucuronyltransferase activity in lung. This was true with both substrates.

F. Comparison of Drug Toxicity and Drug Metabolism in Several Primate and Subprimate Species. It has been recognized for at least five years that looming on the horizon was an impending under-supply of rhesus monkeys for use in biomedical research. Given the heavy reliance that has been placed on the rhesus monkey in drug-development programs and particularly in the development of oncolytic agents, it was deemed desirable to attempt to identify an alternative species to the rhesus for possible use in the Cancer Program. In conjunction with Battelle Columbus Laboratories (BCL) through the Toxicology Program Office (TPO), personnel from the Drug Interactions Unit of the Laboratory of Toxicology devised an experimental design to attempt to identify such a species. Five animal species were selected for study and in order to accomodate possible sex differences, both males and females were scheduled for study. The species were: rhesus monkey, squirrel monkey, tupaia (a primative primate species), mini-pigs and rats. The design involved three major subdivisions, each of which could be pursued independently, yet the data could be finally assembled in a way that all three components could be compared. The subdivisions were: (1) protocol toxicologic evaluation of three selected antineoplastic agents (Adriamycin, Ara C, Cytoxan), (2) in vivo metabolism

...the same ... in drug ... in qualitative differences in drug metabolism), ... in vitro ... of a number of model drug substrates by hepatic preparations ... microsomal and post-microsomal supernatant fractions (to provide data on quantitative differences in drug metabolism). The experiments on points 1 and 2 above were conducted exclusively by BCL employees whereas the experiments in part 3 were conducted jointly by BCL and LT personnel at the BCL facility.

Fourteen parameters of in vitro drug metabolism by hepatic microsomal and soluble fractions were examined in adult male and female rhesus monkeys, squirrel monkeys, Hanford miniature pigs, common tree shrews and Sprague Dawley rats. All species tested demonstrated activity in all but one test assay and all showed some similarity to the rhesus. None of the species, however, was totally comparable to the rhesus in drug metabolizing ability. The squirrel monkey showed the least similarity to the rhesus in drug metabolizing ability, and the miniature pig was the most similar. With the exception of the expected differences in the rat, the tree shrew demonstrated the only sex difference in drug metabolism, the enzyme activities of females being higher than the male in several pathways. The data suggest that any of the four species tested could be a suitable replacement for the rhesus in studies of in vitro drug metabolism. Awaiting completion of these studies, a preliminary impression suggests that the minipig may most closely resemble man in those parameters.

G. Disposition of Cis-diamino-dichloroplatinum (CDDP) in Dogs . In conjunction with the Biomedical Engineering and Instrumentation Branch, Division of Research Services we have investigated the disposition of CDDP following its intravenous injection (1mg/kg) in beagle dogs and rats.

Plasma levels of platinum in dogs showed a distinctly biphasic clearance pattern with estimated half-times of less than one hour and nearly five days. During the first four hours after treatment, plasma levels fell by 90% and 60-70% of the administered dose was recovered in the urine. Platinum was still detectable twelve days after treatment, with no significant change in plasma concentration after the fourth day. Initial concentrations of platinum were highest in organs of excretion, in gonads, spleen and adrenals but remained significantly elevated only in kidney, liver, ovary and uterus, where tissue:plasma ratios of 3 - 4 were maintained for as long as six days post-treatment.

Following IV administration of CDDP to male rats, platinum levels also had a biphasic half-time with an initial phase of ten minutes and small quantities detectable after 48 hours. Large quantities of platinum were recovered in urine during the first 48 hours post-treatment. Organ distribution was similar to that in the dog, with high initial and persisting levels of platinum found in liver, kidney and gonad.

Assay was for total platinum on wet digested tissue extracts using an atomic absorption technique. Binding of the drug to plasma proteins was

demonstrated using standard ultrafiltration techniques and the nature of the binding appeared to change as a function of time.

H. Antineoplastic Agents as Possible In Vitro MFO Substrates. For the past three years, we have examined certain antineoplastic agents for their in vitro metabolism of microsomal enzymes with the aim of finding an agent suitable for routine use for MFO activity. We began our search with compounds that were reported in the literature to be metabolized in vitro and others which we felt had a good potential to be demethylated to yield formaldehyde as one product. We have thus far screened the following drugs: Thalicarpine (NSC68,075), Coralyne sulfoacetate (NSC-154,890), Hexamethylmelamine (13,875), Adriamycin (NSC-123,127), Puromycin (NSC-3,055), Cytembena (NSC-104,801), DTIC (NSC-45,388), Nitidine chloride (NSC-146,397) and 6-Methyl mercaptopurine ribosine (NSC-40,744) and Lidocrine (NSC-247,561). Our results have indicated that the compounds are metabolized, but to a very slight degree (i.e., 50 to 100 times less than our currently utilized substrates). While rechecking literature values we discovered relatively large absolute values (i.e., 30 to 80) but when units were considered (mg/150 mg fresh weight/90 minutes) the specific activity of the metabolic reaction was comparable to our own. Although we will continue to pursue this project, it would appear that the presently available anticancer drugs are poor substrates for in vitro microsomal metabolism.

I. Xenobiotic Distribution and Metabolism in Marine Species. For the past several years we have been exploring the possibility of using the dogfish shark as a model species for the rapid accrual of pharmacokinetic information concerning antineoplastic agents and other xenobiotics. The major rationale for this approach has been that both the rate of metabolism and rate of disposition of compounds administered to these animals might be lower than that of mammals. Within the past three years about 40 drugs and other xenobiotics have been tested in this system. In the past two years, three model compounds of differing dispostion patterns have been studied in depth: (1) Indocyanine green - a non-metabolized drug where hepatic compartment is the sole one; (2) BSP - a drug metabolized by glutathione conjugation and the hepatic compartment is dominant while the renal one is of minor importance; and (3) Phenol red - a drug metabolized by glucuronide conjugation and the renal and hepatic compartments are equally important in its over-all disposition.

(1) In Vivo Distribution and Metabolism. The simultaneous biliary and urinary excretion of a model phenolic compound, phenol red and its major metabolite, a glucuronide, were measured in intact and in biliary fistulized dogfish sharks, Squalus acanthias. It was found that phenol red is extensively metabolized to its glucuronide conjugate by the dogfish shark and that both forms of the drug are actively transported into bile and urine. It was concluded that this species is of excellent potential value in studying the distribution and metabolism of anticancer agents for the following reasons: (1) the dogfish shark is slower to excrete and metabolize xenobiotics - this is very important for antineoplastic

agents because mammals often excrete and detoxify these drugs very rapidly; (2) the hepatic and urinary transport systems of these fish and mammals are qualitatively similar, hence the relative importance of these excretion routes can be rapidly assessed; and (3) this species is abundant, inexpensive and of no economic value.

(2) Pharmacokinetic Studies in Lower Animal Species. In pharmacokinetics, as in other areas of pharmacology, considerably more attention is focused on the extrapolation of results from animals such as rodents, dogs, primates, upwards to man at the top of the phylogenetic hierarchy. Scaling processes also should be applicable in the opposite direction, i.e., from intermediary species to lower species. The validity of pharmacokinetic modeling in aquatic animals has been tested in only very few instances despite the availability of appropriate experimental methodology. Furthermore, there is clearly a need to discover how marine animals in their unique environment handle the increasing number of xenobiotics to which they are exposed. In addition, certain types of intact-animal pharmacokinetic studies may be performed more advantageously in marine species. In order to understand the mechanisms and determine the kinetics of underlying pharmacokinetic phenomena, it is often important to study events occurring in the initial distribution phase for administered agents. In mammalian species the circulation time (blood volume/cardiac output) is typically small compared with practicable sampling intervals. Consequently, in mammals the distribution phase for water-soluble agents often occurs within the first few time points. Resolution may be improved by using a marine species with a relatively longer circulation time.

The present study examined a candidate species, the dogfish shark (Squalus acanthias), which exhibits a circulation time of the order of three minutes. A multicompartment model was utilized to simulate the time course of distribution and excretion of a well-studied model compound, phenol red. A flow-limited multicompartment model was developed to simulate the distribution and disposition of phenol red in the dogfish after intravenous administration. Glucuronide conjugate, as well as parent compound, was found in urine and bile, but not in plasma, kidney, or liver tissue. An apparent four-hour lag in phenol red appearance in the gallbladder was simulated using two stirred tanks connected in series to represent the bile ducts. The model should facilitate use of the dogfish in pharmacokinetic studies of drugs, environmental contaminants, and other xenobiotics.

J. Transport Characterization of Peritoneal Membranes. An animal model is being developed for the intraperitoneal treatment of advanced ovarian cancer with antineoplastic agents. The initial phases of this project involve the study of peritoneal membrane transport of various test compounds by means of radioactive assays and spectrophotometry. We have now determined the characteristics of several compounds varying in molecular size - their absorption and systemic distribution have been elucidated.

Significance to Biomedical Research and the Program of the Institute:

As stated under objectives, drug interactions are known to profoundly influence the disposition of drugs and thus may determine whether a normal therapeutic dose of drug will become either a sub-threshold or toxic dose. The significance of this knowledge to cancer chemotherapy, particularly in the era of combination drug administration, cannot be overemphasized. The studies on peritoneal transport have great potential in the treatment of ovarian cancer.

Proposed Course:

The projects being pursued are only partially complete. In addition to examining in more detail the influence of antineoplastic agents upon the metabolism, distribution, and binding of drugs and their toxicity, we envision studying the metabolic fate of antineoplastic agents. Pharmacokinetic studies of the dogfish shark model have only begun and will continue in greater detail, particularly for those anticancer agents just entering clinical trials.

Publications:

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ANNUAL REPORT OF THE LABORATORY OF CHEMICAL PHARMACOLOGY

NATIONAL CANCER INSTITUTE

NATIONAL INSTITUTES OF HEALTH

July 1, 1975 - June 30, 1976

Studies in the Laboratory of Chemical Pharmacology are concerned with the rational design of antitumor agents and with elucidation of the pharmacological properties of established and potential antitumor agents. These include the disposition, mechanism of action, structure-activity relationships, toxicity reversal, and dose-response relationships of antitumor drugs and other model foreign compounds in laboratory animals and man. In many instances these studies require the development of new methodologies to facilitate pharmacologic studies of antineoplastic agents whose disposition is not fully understood. Of special interest in disposition studies is the establishment of rates and rate constants which govern the passage of drug across membranes from one body compartment to another, the assessment of drug biotransformation or binding to cellular constituents or macromolecules and the determination of the rate of elimination of the drug and its metabolites. These studies are important since one of the major factors contributing to an inadequate drug response in the host is the failure of the drug to reach the target site in sufficient concentration to eradicate the tumor cells. The knowledge gained from such studies when considered together with biochemical, toxicologic, and cytokinetic data should enable the clinician to utilize drug dosages and schedules for the most effective treatment of human malignancies.

In addition, another function of the laboratory is to develop methods for the determination of specific products of tumor cells in experimental animals and man. The analysis of such products in body fluids would be valuable for diagnostic purposes and for the assessment of the antitumor effects of various therapeutic modalities.

During the past year several research projects were continued and a number of new studies were initiated. Some of the experimental findings are summarized below.

Further studies on the folic acid antagonists were performed both in animals and humans. Studies were conducted in mice in which plasma concentration of methotrexate (MTX) were maintained constant for prolonged periods of time with a mini-infusion device. Two lines of investigation were pursued. First, the effects of concentration and exposure time of MTX *in vivo* on the normal mouse bone marrow were studied by utilizing an *in vitro* bone marrow colony assay. Second, the effects of MTX *in vivo* were investigated by studying incorporation of labeled precursors into DNA of cells from the Lewis lung tumor, small intestine and bone marrow.

By using the *in vitro* technique it was shown that MTX toxicity to bone marrow resulted from inhibition of both purine and pyrimidine synthesis and could be reversed in a non-competitive fashion by addition of thymidine and a purine source. *In vivo* studies using a constant infusion device demonstrated that higher levels of MTX produced more rapid depletion of bone marrow but the maximum cell kill was the same for all levels of MTX above 10^{-8}M. When *in vivo* infusion studies were performed using various levels of MTX it was found that the degree of inhibition of nucleoside incorporation into DNA was: intestine > bone marrow > Lewis lung tumor. The greater resistance of the Lewis lung tumor to MTX was due to the fact that the DHFR activity per mg of protein was about six times as great in the tumor relative to small intestine and the growth fraction was about one quarter of the small intestine. Both of these factors would naturally confer resistance to MTX.

Studies indicated that bone marrow *in vivo* resembles bone marrow *in vitro* with respect to rescue with calcium leucovorin and that the plasma concentration ratio between calcium leucovorin and MTX was critical in determining the rescue effect of citrovorum factor. Plasma MTX levels in patients are being quantitated at intervals after treatment in order to assess the need for continued leucovorin rescue measures, and the value of such monitoring has already been demonstrated. Plasma MTX levels below 5×10^{-7}M at 48 hours after treatment are not associated with drug toxicity, and leucovorin rescue measures can be safely discontinued at this plasma concentration. During the past year over 125 MTX infusions have been monitored, and no significant toxicity was demonstrated when plasma levels were 5×10^{-7}M or less.

Studies of the disposition and myelotoxicity of methotrexate in patients with carcinoma of the ovary were continued and the threshold for inhibition of DNA synthesis in bone marrow as previously reported was $\sim 1\text{-}3 \times 10^{-8}$M. The phenomenon of preferential accumulation of MTX in ascites was investigated using pharmacokinetic modeling and appeared to be the result of limited diffusion capacity of the peritoneal surface in relation to the volume of ascites. MTX binding to ascites and plasma has been investigated and appears to be a constant fraction of total drug regardless of drug concentration.

Preliminary testing of Carboxypeptidase G_1 in man was undertaken in 1973-1974, and indicated the ability of this enzyme to deplete circulating folates and to cleave circulating methotrexate; one instance of hypersensitivity in man was also found. The main effort at present is to obtain sufficient enzyme to investigate its utility as a rescue agent following high dose methotrexate in humans. In addition, the enzyme has proved valuable as a means of identifying α-peptide glutamate groups in folate analogs such as metabolites of methotrexate esters and polyglutamates.

Disposition studies in laboratory animals were continued for three anticancer agents [coralyne sulfoacetate (NSC-154980), an acridine derivative (NSC-141549), and inosine dialdehyde (NSC-118994)]. In addition, studies were initiated in laboratory animals for three new agents [3-deazauridine (NSC-126849), spirohydantoin mustard (NSC-172112) and chlorozotocin (NSC-178248)]. The pharmacokinetics of gallium were continued in humans in conjunction with

an ongoing phase I clinical trial.

The disposition of [^{14}C]-3-deazauridine was studied in normal and L1210 tumored mice following an intraperitoneal injection at the optimal therapeutic dose (300 mg/kg). The compound is rapidly eliminated in the urine (80% in 4 hours) as unchanged 3-deazauridine. Administration of [^{14}C]-3-deazauridine with the radioactive label in either the ribose or the pyridine moiety and subsequent chromatographic analysis demonstrated that the glycosidic linkage is not disrupted *in vivo*. 3-deazauridine is phosphorylated *in vivo* by all tissues to the corresponding nucleoside mono-, di-, and triphosphate forms. Tissue concentrations of 3-deazauridine and its nucleotide forms were determined by ion exchange column chromatography at intervals of 30', 60', 120', 240' and 24 hours after injection. The compound was widely distributed in all tissues, including brain. At early time intervals, highest concentrations were found in kidney, probably because urinary excretion was the primary route of elimination. A compariso of plasma and tissue levels as a function of time indicated that 3-deazauridine (nucleoside) readily equilibrates between plasma and tissues; however, following phosphorylation, passage from tissues to plasma is limited and 3-deazauridine accumulates intracellulary as the nucleotide. Therefore, the level of uridine kinase in the various tissues is likely to be a critical factor in the tissue distribution at later time intervals and the tissue distribution of this enzyme is presently being studied.

Chlorozotocin is a nitrosourea analog with antitumor activity but without severe bone marrow toxicity in mice. It can be measured in µg quantities by a modified Bratton-Marshall reaction (nitroso group) and by the NBP reaction (alkylating group). The former procedure has been used in preliminary *in vitro* studies of its hepatic metabolism. About 50% of the drug was destroyed non-enzymatically after a 30 min incubation at 37°. The extent of destruction of the nitroso group was not enhanced by homogenates or microsomal preparations of rat liver. *In vivo* studies are in progress with chlorozotocin-[1-^{14}C-glucose] and chlorozotocin-[2-^{14}C-ethyl]. Following i.v. administration of the glucose label to a limited number of rats, 45% of the radioactivity was eliminated in the urine in 6 hr, and 5% in the bile. In contrast, approximately 35% of the radioactivity after administration of the ethyl label appeared in the urine in 6 hr, and 35% in the bile. The portion of chlorozotocin-[2-^{14}C-ethyl] eliminated in the bile fell rapidly and by 20 min the majority of radioactivity was associated with 2 metabolites. The plasma decay curve of both labels showed an initial rapid phase followed by a slow phase. Five to ten min after administration of the drug, approximately 2% of the radioactivity in the plasma was in bound form, whereas at 6 hr 60% of the glucose label and 80% of the ethyl label were bound. Disposition studies will continue in rodents and monkeys prior to clinical evaluation and will provide the framework for the design of phase I clinical trials.

Spirohydantoin mustard is an alkylating agent with activity against intracranially-implanted ependymoblastoma. Levels of 1 µg can be measured colorimetrically by the NBP reaction; with this method, the *in vitro* half-life of alkylating activity in mouse serum is 5-6 min at 37°. A more sensitive assay procedure, capable of detecting less than 1 ng of parent

drug in vivo, has been developed and involves extraction of drug into ethyl acetate, gas-liquid chromatography and mass spectrometry with selected ion monitoring of m/e 154 and 156. Studies on the disposition of this agent will soon begin in the dog and monkey.

Pharmacokinetic studies of gallium, using Ga^{67} as a tracer, are under investigation in humans in conjunction with a phase I study of this agent. Plasma disappearance curves are bipnasic, with an initial rapid component followed by a much more gradual decline. Excretion is primarily via the urinary route with 26 to 32% eliminated during the first 24 hours; 2.2 to 5.4% is eliminated in the feces during the first 48 hours.

An antitumor agent carried selectively by lymphatic channels would be likely to have therapeutic advantages in treating lymphoid malignancies and tumors which metastasize by lymphatics. Studies were undertaken to examine the extent to which clinically effective antitumor agents were absorbed and distributed via the lymphatics as well as to define the chemical and/or structural characteristics required for the selective uptake of compounds by lymphatics. In achieving this objective, it would be possible to design antitumor agents for selective lymphatic absorption. The lymphatic absorption and biliary and urinary excretion of 12 radioactively labelled antitumor agents was examined in the rat. The compounds studied were ^{14}C-CCNU, ^{14}C-methyl-CCNU, ^{14}C-guanazole, ^{14}C-dibromomannitol, ^{14}C-diethylstilbestrol, ^{3}H-prednisolone, ^{3}H-dexamethasone, ^{14}C-6-mercaptopurine, ^{14}C-cytosine arabinoside, ^{3}H-vincristine, ^{14}C-cyclophosphamide and ^{14}C-o,p-DDD. Results to date indicate that only ^{14}C-o,p-DDD is extensively absorbed via the lymphatics. Urinary excretion was the major route of elimination for ^{14}C-guanazole, ^{14}C-dibromomannitol, ^{14}C-6-mercaptopurine, ^{14}C-cyclophosphamide, and ^{14}C-cytosine arabinoside; ^{14}C-diethylstilbestrol, ^{3}H-prednisolone, ^{3}H-dexamethasone and ^{3}H-vincristine were largely excreted via the bile.

Studies designed to elucidate the molecular basis of cytotoxicity for several new anticancer agents were performed. The acridine derivative NSC-141549 (MeAc) was tested for inhibition of reverse transcriptase activity of avian myeloblastosis virus, DNA polymerase activity of NIH-Swiss mouse embryo, and RNA polymerase activity of E. coli. The viral and cellular enzyme activities were strongly inhibited by MeAc when template-primers of A:T base pairs were used. However, no inhibition of reverse transcriptase activity was obtained with poly rC·oligo dG suggesting that the drug interacts specifically with A:T base pairs. This conclusion was corroborated by studying the inhibition of E. coli RNA polymerase by MeAc with different templates of known composition. Furthermore, the thermal stability of synthetic double-stranded nucleic acid polymers containing A:T base pairs was enhanced by MeAc whereas polymers containing only G:C base pairs exhibited no change in their thermal stability. The thermal stability of calf thymus DNA was only slightly increased by MeAc indicating that the primary base sequence is also an important factor in MeAc intercalation.

An analog of uridine, 3-deazauridine (NSC-126849) was found to be phosphorylated in vivo to the corresponding nucleotides by mouse tissues and ascites L1210 cells. The in vivo phosphorylation rates were lower in normal cells than in L1210 cells where 3-deazauridine triphosphate was accumulated. Furthermore, 3-deazauridine was not ultimately incorporated into cellular DNA or RNA which probably accounts for the accumulation of 3-deazauridine triphosphate. It appears that the cytotoxicity of 3-deazauridine derives from its triphosphate form which may inhibit nucleic acid polymerases; alternatively it may function as a fraudulent allosteric inhibitor of uridine kinase, an enzyme critical for the control of cellular differentiation and division.

Inosine dialdehyde (IdA), an antitumor agent undergoing phase II clinical trial, was found to cross-link plasma proteins in vitro. This interaction might explain the increased thrombin time reported for patients receiving IdA. IdA interacts with RBC membranes irreversibly under physiologic conditions and a cross-linking of plasma proteins with RBC membranes in vivo could result in the positive Coomb's reaction exhibited by patients receiving IdA. The interaction of IdA with proteins can be inhibited by the addition of small molecules containing primary amino groups (e.g., amino acids). These small molecules can also reverse the in vitro cytotoxicity of IdA. The use of such small molecules might prove beneficial in controlling the clinical toxicities of IdA.

Cytosine arabinoside 5'-methylphosphonate (ara-CMeP) was designed and synthesized as an analog of ara-CMP in an attempt to circumvent resistance to araC. This compound is a competitive inhibitor of dCMP kinase but is not phosphorylated by this enzyme. This suggests that ara-CMeP can bind as well as ara-CMP to dCMP kinase because the pyrimidine moiety confers sufficient structural similarity to ara-CMP; however, methylation of the phosphate moiety alters the nucleophilicity of this portion of the molecul such that phosphorylation does not occur. These results are interesting in that they expand our knowledge of mechanisms of enzyme catalysis, but also indicate that this type of structural alteration might not lead to compounds that would be successful as antitumor agents.

Previously initiated studies on enzymes involved in the metabolism of antineoplastic cytidine analogs have been continued. Cytidine deaminase, deoxycytidine kinase and uridine-cytidine kinase have been characterized and purified several hundred fold using human tissue or human tissue cultu leukemia lines as an enzyme source. Of interest was the finding that uridine-cytidine kinase, the enzyme which converts 5-azacytidine to 5-aza- cytidine phosphate, had a low affinity for 5-azacytidine. This low affinity as well as the low enzyme levels found in CEM cells, suggest that alternative explanations for the activation and cytotoxic effects of 5-azayctidine be investigated.

Cyclic AMP is thought to be involved with cellular morphology growth, and differentiation. Studies were therefore initiated to determine whether tumor cells contain cyclic nucleotide phosphodiesterase, the enzyme which catalyzes the hydrolysis of cyclic AMP, and to elucidate the mechanism of activation of this enzyme. Thus far cyclic nucleotide phosphodiesterase

and a heat stable Ca^{++} dependent non-dialyzable protein activator have been found in various animal and human cancer cells in culture. The relationship of this enzyme and its activator to cell transformation, malignancy and chemotherapy is under study.

The antitumor activity of several groups of compounds including maytansine analogs, adenosine analogs, group IIIa metals and their salts, immunostimulants, interferon and some rationally designed compounds is being evaluated in various experimental rodent tumors.

New antitumor agents and those in clinical use are being evaluated for embryotoxic, cytogenetic and carcinogenic effects. Comparative studies of the embryotoxicity and cytogenetic effects in mice of a series of antitumor agents known to be spindle poisons are in progress. The agents under study are vincristine, maytansine, colchicine and two epipodophyllotoxin derivatives (VP-16 and VM-26).

The availability of a large breeding and experimental monkey colony (600 animals representing 5 species) continues to provide this laboratory and other cooperating units with a unique resource for comparative pharmacologic, toxicologic, and biochemical studies in nonhuman primates. Various antitumor agents, including adriamycin, procarbazine, and melphalan are being tested for carcinogenic activity in long-term studies in monkeys. A total of 51 monkeys have been treated with procarbazine for periods ranging from 1 to 10 years. To date, eight monkeys (16%) have developed a malignancy, and 5 of the 8 neoplasms were hamatologic (acute leukemia). To date none of the monkeys treated with adriamycin or melphalan have developed tumors but these studies have only been carried out for 12 months or less. Six other chemicals have induced tumors in three species of Old World monkeys under study - these include 3 nitrosamines: [diethylnitrosamine (DENA), 1-nitrosopiperidine (PIP) and dipropylnitrosamine (DPNA)], methynitrosourea, cycasin and its aglycone (MAM-acetate), and aflatoxin B_1. Methylnitrosourea has induced squamous cell carcinomas of the mouth, esophagus and pharynx; MAM acetate has induced primary liver cell carcinoma and carcinoma of the kidneys; and the nitrosamines and aflatoxin B_1 have induced primary liver cell carcinomas in Old World monkeys. Alpha-fetoprotein (AFP) levels in sera of monkeys with biopsy-proven tumors were measured using radioimmunoassay. The wide range of values (100,000-fold variation) was analogous to that seen in patients with hepatocellular carcinoma. Monkeys with DENA-induced neoplasms had significantly higher serum levels of AFP than those with tumors induced by 1-nitrosopiperidine, MAM acetate or aflatoxin B_1. Serial determinations of AFP levels in monkeys with liver tumors demonstrated that the values tended to plateau after reaching a level characteristic for that tumor.

An analysis of the composition of isolated tRNA's in livers of normal and hepatoma-bearing monkeys, quantitation of tRNA degradation products in urine, and an assessment of the methylating ability of individual tRNA methyltransferases has been carried out. Although only minor variations in the base composition of tRNA's from normal liver and hepatoma tissue were detected, increased excretion of pseudouridine, methylated nucleosides and bases, and β-aminoisobutyric acid were found in urine from hepatoma-bearing

monkeys, indicating an increased tRNA turnover rate in hepatoma tissue. Methyltransferase activity for the hepatoma-bearing monkeys was higher than in normal monkeys; wide variations in individual methylase activities were found, with hepatoma-bearing monkeys having higher levels of thymine, and 7-methylguanine, and lower levels of N^2,N^2-dimethylguanine. These results, along with the increased urinary excretion of methylated nucleosides and bases in hepatoma-bearing monkeys suggest aberrant methylation of tRNA, while results from studies of tRNA composition indicate increased tRNA turnover.

In addition to the basic laboratory research effort, each senior investigat shares the responsibility for the administration and supervision of one or more contracts with outside research institutions. The contracts are primarily concerned with development of analytical-biochemical methodology; the study of pharmacological disposition of antitumor drugs and their metabolites in humans and laboratory animals; the development of pharmacokinetic mathematical models; and the study of biological markers for detection of cancer and assessment of cancer treatment. Approximately 10 new antitumor agents are studied intensively each year, reflecting the interest of the Division of Cancer Treatment Program area in developing clinically effective antitumor drugs.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03502-13 LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Folic Acid Antagonists in Experimental Solid Tumors and Normal Tissues of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	R. L. Dedrick	Chemical Engineer	BEIB DRS
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COOPERATING UNITS (if any)

Medicine Branch, NCI; Laboratory of Toxicology, NCI; and Biomedical Engineering and Instrumentation Branch, DRS

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacokinetics and Pharmacodynamics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	None

SUMMARY OF WORK (200 words or less - underline keywords)

Constant infusions of MTX into mice have been conducted in order to provide us further understanding on organ specificity with respect to rate of inhibition and rate of recovery of certain biochemical pathways and cell growth. In vivo exposure to drug has been followed by in vitro cloning of nucleated cells to determine bone marrow viability at different stages of drug exposure and of drug removal. In vitro colony forming ability of normal bone marrow has been tested with combinations of MTX and several rescue agents (calcium leucovorin, thymidine, hypoxanthine, inosine, guanosine and adenosine).

In vivo rate of inhibition and rate of recovery of certain biochemical pathways in small intestine, bone marrow and Lewis lung tumor after constant infusions of MTX have been studied. Changes in recovery rates of these biochemical pathways in the three different organs have been studied following administration of a variety of rescue agents as listed above.

Project Description:

Studies in mice have been conducted in which plasma concentration of methotrexate have been maintained constant for prolonged periods of time with a mini-infusion device developed through a cooperative effort with Drs. Dedrick and Lutz of BEIB. Two directions have been pursued. Dr. Pinedo investigated the effects of concentration and exposure time of MTX in vivo on the normal mouse bone marrow by utilizing an in vitro bone marrow colony forming assay conducted in cooperation with the Medicine Branch. We have continued monitoring the effects of MTX directly in vivo by studying the incorporation of labeled precursors into DNA of the Lewis lung tumor, small intestine and bone marrow.

The in vitro cloning studies have resulted in several interesting observations. Bone marrow toxicity is both a function of methotrexate concentration and duration of exposure. For example, a MTX plasma concentration of 1×10^{-5}M reduced the surviving fraction of nucleated cells per femur to 30% of control when the mice were exposed for only 12 hours. However, an exposure of 65 hours at only 5×10^{-8}M MTX resulted in the same depletion but it took longer. This suggests a longer persistent depletion of nucleated cells at high concentrations and hence a reason for enhanced toxicity. It was also shown that recruitment of bone marrow colony forming cells was a function of the time at which the nucleated cells in the femur remained at the depleted level of 30% of control. Such recruitment indicates the need for caution in timing of subsequent drug administration in order to avoid extreme toxicity as a result of hitting a large proportion of dividing cells.

Preliminary trials attempting to block colony formation in vitro from normal bone marrow with MTX were unsuccessful. Studies using high pressure liquid chromatography (HPLC) indicated the presence of substantial amounts of nucleosides in both fetal calf serum and L-cell extract, both necessary medium growth factors. Dialysis of these substances removed these nucleosides and permitted clone formation which then could be inhibited by MTX. This then enabled studies to be conducted with rescue factors such as calcium leucovorin, thymidine and various purines. The results indicate that calcium leucovorin competes with MTX to prevent its toxic effects. An equal molar concentration of calcium leucovorin is needed to prevent toxicity at 1×10^{-8}M MTX whereas at 1×10^{-6}M MTX at least 1×10^{-4}M calcium leucovorin is needed. At 1×10^{-3}M, calcium leucovorin precipitates out in the medium due to the presence of salts and therefore rescue of MTX toxicity when the drug is maintained above 1×10^{-6}M is not possible.

The use of thymidine and purines as rescue agents in vitro indicated that in normal bone marrow neither pyrimidine alone nor purine alone at any concentration can reverse toxicity. It was necessary to have

thymidine plus a purine other than guanosine (e.g., hypoxanthine, adenosine or inosine) to effect rescue. The range of effective nucleoside concentration for effective rescue was very small, between 5×10^{-6}M and 5×10^{-5}M thymidine. Little effect was seen below 5×10^{-6}M and toxic effects appeared above 1×10^{-5}M.

The in vivo studies on small intestine, bone marrow and Lewis lung tumor are being conducted with a similar approach but by their nature take longer to accumulate data. Infusion studies at various levels of MTX have shown that degree of inhibition of nucleoside incorporation into DNA is in the following order: intestine > bone marrow > Lewis lung tumor. The greater resistance of the Lewis lung tumor to MTX is due to several factors. It is not due to increased binding of MTX to the enzyme dihydrofolate reductase (DHFR) since the I_{50} for equivalent enzyme activity from bone marrow, small intestine and Lewis lung tumor are very similar. However, the DHFR activity per mg of protein is about six times as great in the tumor relative to small intestine and the growth fraction is about one quarter of the small intestine. Both of these factors would naturally confer resistance to MTX. The rate of recovery of femur marrow and Lewis lung tumor from MTX inhibition of nucleoside incorporation into DNA is approximately twice as fast. This suggested to us the more efficient utilization of salvage metabolites by these organs from their blood supply in vivo. Investigations are currently being conducted using calcium leucovorin, thymidine and purines delivered in vivo with constant infusions to test this hypothesis. Preliminary data with calcium leucovorin verify that recovery of femur marrow and tumor is much more rapid than small intestine.

We have also collected data which indicates that bone marrow in vivo acts like bone marrow in vitro with respect to rescue with calcium leucovorin. The results also indicate that the plasma concentration ratio between calcium leucovorin and MTX is critical in determining the rescue effect of citrovorum factor and that this relationship varies amongst the three tissues studied.

Publications:

1. Dedrick, R. L., Zaharko, D. S., Bender, R. A., Bleyer, W. A. and Lutz, R. J.: Pharmacokinetic considerations on resistance to anticancer drugs. Cancer Chemother. Rep. 59: 795-804, 1975.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 03505-13 LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Potential Embryotoxic, Carcinogenic and Other Adverse Effects of Anticancer Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
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SECTION
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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

New antitumor agents and those in clinical use are being evaluated for embryotoxic, cytogenetic and carcinogenic effects. Comparative studies of the embryotoxicity and cytogenetic effects in mice of a series of antitumor agents known to be spindle poisons are in progress. The agents under study are vincristine, maytansine, colchicine and two epipodophyllotoxin derivatives (VP-16 and VM-26). Various antitumor agents, including adriamycin, procarbazine, azathioprine and melphalan are being tested for carcinogenic activity in long-term studies in monkeys. The medical literature as well as patient data at the NCI continues to be monitored in order to gain more information as to the carcinogenic potential in humans of clinically-used antitumor agents. Other adverse effects of these agents are also continuously being tabulated from the literature, including teratogenic, mutagenic and cytogenetic effects.

PHS-6040

Project Description:

Objectives:

This project is designed to obtain data on various aspects of the toxicity of antitumor agents, including their embryotoxic, cytogenetic and carcinogenic effects in rodents, monkeys and in the human. Specifically, the objectives are:

To compare in mice the embryotoxic and cytogenetic effects of a series of mitotic inhibitors with antitumor activity, including both established drugs (vincristine and colchicine) as well as new agents (maytansine and epipodophyllotoxin derivatives).

To evaluate in mice and in monkeys the carcinogenic potential of various clinically-used antitumor agents administered singly and in combinations with other agents and/or irradiation.

To monitor the current medical literature and the patient data at the NCI in order to tabulate and characterize adverse effects of antineoplastic agents in man. Particular emphasis is being given to embryotoxic, mutagenic and carcinogenic effects arising from treatment with clinically effective antitumor agents.

Major Findings:

Embryotoxicity of Antitumor Agents

Comparative studies on the embryotoxic and cytogenetic effects of a group of antitumor agents known to be spindle poisons (vincristine, maytansine, colchicine, and two epipodophyllotoxin derivatives - VM-26 and VP-16) are in progress. Our results to date indicate that vincristine is markedly embryolethal when administered to pregnant mice on day 6, 7 or 8 of gestation, killing 35%, 81% and 78% of fetuses/litter, respectively. Equimolar doses (0.36 μmoles/kg) of maytansine are also embryolethal, killing 21%, 57% and 48% of fetuses/litter when given on gestational day 6, 7 and 8, respectively. Colchicine has significant embryolethal activity during this gestational period, but only at 10-fold higher dose levels. Vincristine and maytansine both exert teratogenic effects in mice on day 7 of gestation. At equimolar doses (0.36 μmoles/kg), 90% of fetuses/litter were malformed after treatment with vincristine, whereas this figure was 63% for maytansine. In contrast to maytansine, vincristine was also teratogenic when given on day 6 of gestation, but neither compound exerted significant teratogenic activity when administered on gestational day 8. Althouth colchicine was teratogenic when given on gestational days 6 or 7, this effect was noted only at doses 10-fold higher than those required to elicit teratogenic effects from vincristine or maytansine. During the course of these studies, an important difference between vincristine and maytansine was noted. The optimal antitumor dose for vincristine against a number of experimental

tumor systems (0.25 mg/kg) was equal to the dose at which a maximal teratogenic effect was noted. In contrast, the optimal antitumor dose for maytansine was 25 µg/kg, whereas its embryotoxic and teratogenic effects were noted only at doses some 10-fold higher. Thus, the difference between the antitumor dose and the embryotoxic dose is far greater for maytansine than for vincristine.

Cytogenetic studies of samples of maternal bone marrow and fetal liver tissue indicate that the mitotic index is increased following treatment with either vincristine or maytansine, although there is not a statistically significant increase in the incidence of numerical or structural chromosomal aberrations. A comparison of the karyotypes of normal control fetuses or morphologically normal vincristine- or maytansine- treated fetuses with those of morphologically abnormal drug-treated fetuses did not reveal significant differences in number of structural or numerical chromosomal aberrations.

Evaluation of Antitumor Agents for Carcinogenic Activity

Procarbazine, alone and in combination with x-irradiation, is carcinogenic in mice and rats. A total of 51 monkeys have been treated with procarbazine for periods ranging from 1 to 10 years. To date, eight monkeys (16%) have developed a malignancy, and 5 of the 8 neoplasms were hematologic (acute leukemia). Procarbazine may also have carcinogenic activity in man. A number of Hodgkin's disease patients successfully treated with the MOPP regimen (nitrogen mustard, vincristine, procarbazine and prednisone) plus irradiation have developed a second malignancy, most frequently acute myelogenous leukemia. Although procarbazine is a component of the MOPP regimen, it cannot with certainty be implicated as the causative factor in the second malignancies developing in Hodgkin's disease patients, the laboratory data notwithstanding. A high frequency of acute myelogenous leukemia has also been noted among patients treated with melphalan for multiple myeloma. This observation is of particular importance in view of the increasing enthusiasm for adjuvant treatment with melphalan following surgery for breast or ovarian cancer. To date, none of the 20 monkeys treated with melphalan have developed a malignancy, although treatment with this drug has only been carried out for a maximum of 12 months.

Publications:

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2. Adamson, R. H., McIntire, K. R., Sieber, S. M., Correa, P., and Dalgard, D. W.: Non-human primate models for lymphoma, leukemia and other neoplasms. In Comparative Leukemia Research, 1973. Tokyo/Karger, Basel, University of Tokyo Press, 1975, pp. 723-730.

3. Sieber, S. M., and Adamson, R. H.: Maintenance therapy in myeloma: risk versus benefit. Br. Med. J. 2: 557, 1975.

4. Sieber, S. M.: Cancer chemotherapeutic agents and carcinogenesis. Cancer Chemother. Rep. 59: 915-918, 1975.

5. Sieber, S. M., and Adamson, R. H.: Toxicity of antineoplastic agents in man: chromosomal aberrations, antifertility effects, congenital malformations and carcinogenic potential. Adv. Cancer Res. 22: 57-155, 1975.

6. Sieber, S. M.: The action of antitumor agents: a double-edged sword? Med. Pediatr. Oncology, in press.

7. Sieber, S. M., Wolpert, M. K., Adamson, R. H., Cysyk, R. L., Bono, V. H., and Johns, D. G.: Experimental studies with maytansine - a new antitumor agent. Proc. VII Int. Symp. Comp. Res. Leuk. Related Dis., in press.

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PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 03506-13 LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pharmacology and Disposition of Antitumor Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

None

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SECTION
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NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Pharmacologic disposition studies are in progress for several new antitumor agents. These compounds are either in early stages of clinical evaluation or are under consideration for clinical trial. By selecting compounds early in their development, information as to their distribution, metabolism, absorption, etc. can be made available to clinicians along with recommendations to aid in the design of the clinical trial. Such information is essential for a successful clinical evaluation of the agent.

Studies in laboratory animals were continued (see previous report) for three agents [coralyne sulfoacetate (NSC-154980), an acridine derivative (NSC-141549), inosine dialdehyde (NSC-118994)]. Studies were initiated for three additional agents in laboratory animals [3-deazauridine (NSC-126849), spirohydantoin mustard (NSC-172112) and chlorozotocin (NSC-178248)]. In addition, the pharmacokinetics of gallium were continued in humans in conjunction with phase I clinical trial.

PHS-6040
(12-75)

Project Description:

Objectives:

To study the metabolic fate of experimental antitumor agents prior to clinical trial and to ascertain whether their metabolites are active in various tumor systems.

To study the pharmacology of antitumor drugs of current interest in clinical chemotherapy in order to design better therapeutic regimens.

To study the clinical pharmacology of new agents in phase I clinical trial.

To evaluate the usefulness of new tumor systems in experimental chemotherapy.

To design newer anticancer drugs by rational approaches.

Methods Employed:

Usual pharmacologic, biochemical, analytical, and immunologic techniques.

Major Findings:

Disposition of NSC-141549 (MeAc), Methanesulfon-m-Anisidide, 4'-(9-Acridinyl-9-Amino) Monohydrochloride

Disposition studies of ^{14}C-MeAc were extended (see previous annual report). A procedure was developed for selectively extracting unchanged MeAc from biologic materials. Following intraduodenal administration to rats, ^{14}C-MeAc is rapidly absorbed but immediately excreted in the bile as a metabolite, with only trace amounts of radioactivity reaching the systemic circulation. An intraduodenal dose of 100 mg/kg was found to saturate the system that actively transports the metabolite of MeAc into the bile. The results indicate that oral administration of MeAc would provide high levels of drug in the liver with a minimum amount reaching the systemic circulation; a phenomenon that suggests the use of this agent orally for liver tumors. The major metabolite of MeAc is thought to be a conjugate of glutathione and the acridine ring. The 9-position of MeAc is vulnerable to attack by a variety of nucleophils and the reaction can occur non-enzymatically *in vitro*. The very rapid rate of MeAc metabolism may be essential for the selective toxicity exhibited by this agent. Studies with rat liver glutathione S-transferase demonstrated that MeAc inhibits non-competitively with respect to glutathione or dichloronitrobenzene. MeAc has a high affinity for pigmented tumors. Concentrations of MeAc in s.c. inplanted B_{16} melanoma were 10 to 20 fold higher than in s.c. implanted L1210 following i.p. administration. The interaction of MeAc with melanosomes is presently under study to determine if the affinity of MeAc for pigmented tissue may be exploited clinically.

Disposition of NSC-126849 (3-Deazauridine)

The disposition of [^{14}C]-3-deazauridine was studied in normal and L1210 tumored mice following the optimal therapeutic dose in mice (300 mg/kg i.p.). The compound is rapidly eliminated in the urine (80% in 4 hours) as unchanged 3-deazauridine. Administration of [^{14}C]-3-deazauridine with the radioactive label in either the ribose or the pyridine moiety and subsequent chromotographic analysis demonstrated that the glycosidic linkage is not disrupted. 3-deazauridine is phosphorylated in vivo by all tissues to the corresponding nucleoside mono-, di-, and triphosphate forms. Tissue concentrations of 3-deazauridine and its nucleotide forms were determined by ion exchange column chromatography at intervals of 30', 60', 120', 240' and 24 hours after injection. The compound was widely distributed in all tissues, including brain. At early time intervals, highest concentrations were found in kidney, probably as a result of urinary excretion being the primary route of elimination. A comparison of plasma and tissue levels as a function of time indicated that 3-deazauridine (nucleoside) readily equilibrates between plasma and tissues; but following phosphorylation, passage from tissues to plasma is limited and 3-deazauridine accumulates intracellulary as the nucleotide. Therefore, a critical factor in the tissue distribution at later time intervals would be the level of uridine kinase in the various tissues. A tissue distribution of uridine kinase is presently being studied. No differences in tissue distribution or tissue phosphorylation rates could be detected between male and female mice, although a previous report indicated 3-deazauridine to be more toxic in female mice. The tissue distribution of ^{14}C-uridine (300 mg/kg) was similar to that of 3-deazauridine. The disposition of 3-deazauridine at two lower doses (150 and 75 mg/kg) is presently under study.

Clinical Pharmacology of Gallium

The pharmacokinetics of gallium is under investigation in humans using Ga^{67} as a tracer. This is in conjunction with the phase I study of this agent. Plasma disappearance curves were found to be biphasic with an initial rapid component followed by a much more gradual decline. Excretion was primarily via the urinary route with 26 to 32% eliminated during the first 24 hours while 2.2 to 5.4% was eliminated in the feces during the first 48 hours.

Studies on Spirohydantoin Mustard (NSC-172112)

Spirohydantoin mustard is an alkylating agent with activity against i.c. implanted epdenymoblastoma. It can be measured colorimetrically by the NBP reaction to levels of 1 μg. Using this method, the in vitro half-life for serum decay of alkylating activity is 5-6 min at 37°. A more sensitive assay procedure has been developed which enables detection of parent drug in vivo. The procedure involves extraction of spirohydantoin mustard with ethyl acetate, gas-liquid chromatography, and mass spectrometry with selected ion monitoring of m/e 154 and 156. Using an internal standard,

quantitative analysis of less than 1 ng of drug injected on the column is possible. Studies on the disposition of this agent will soon commence in the dog and monkey.

Disposition of Coralyne Sulfoacetate (NSC-154890)

Disposition studies of [^{14}C] coralyne sulfoacetate in the rat were continued. The plasma disappearance curve of total radioactivity showed at least two components, both of which were affected by dose. Following i.v. administration of 13, 26 and 52 mg/kg doses, the plasma half-lives obtained for the initial distribution phase were 12.3, 16.2 and 21.4 min, respectively, and those for the second phase were 236, 179 and 146 min, respectively. Twenty-four hours after administration, radioactivity in the tissues was concentrated in the kidney (2.5 to 9 times greater specific activity than the liver). As urinary excretion is only 1% in 24 hr, this concentration in the kidney may be due to strong protein binding. Coralyne does bind significantly to rat albumin with an association constant of 1.2 x 10^5M for coralyne concentrations below 2 x 10^{-5}M. At higher concentrations binding occurs but the affinity is greatly reduced.

Disposition and Metabolism of Chlorozotocin (NSC-178248)

Chlorozotocin is a nitrosourea analog with antitumor activity and low bone marrow toxicity in mice. It can be measured in µg quantities by a modified Bratton-Marshall reaction (nitroso group), and by the NBP reaction (alkylating group). The former procedure has been used in preliminary studies to investigate hepatic metabolism of the drug *in vitro*. When 1.5-10 µmoles of chlorozotocin were incubated aerobically and anaerobically about 50% of the drug was destroyed non-enzymatically within 30 min at 37°. The destruction of the nitroso group was not enhanced by homogenates or microsomal preparations of rat liver. *In vivo* studies are in progress with chlorozotocin-[1-^{14}C-glucose] and chlorozotocin-[2-^{14}C-ethyl]. Following i.v. administration of the glucose label to a limited number of rats, 45% of the radioactivity was eliminated in the urine in 6 hr, and 5% in the bile. In contrast, approximately 35% of the ethyl label appeared in the urine in 6 hr, and 35% in the bile. The portion of chlorozotocin-[2-^{14}C-ethyl] eliminated in the bile fell rapidly and by 20 min the majority of radioactivity was associated with 2 metabolites. The plasma decay curve of both labels showed an initial rapid phase followed by a slow phase. Five to ten min after administration of the drug, approximately 2% of the radioactivity in the plasma was in bound form, whereas at 6 hr 60% of the glucose label and 80% of the ethyl label were bound.

Publications:

1. Adamson, R. H.: Pharmacology of newer antineoplastic agents. In Sartorelli, A. C., and Johns, D. G. (Eds.): Antineoplastic and Immunosuppressive Agents II. New York, Springer-Verlag, 1975, pp. 877-890.

2. Adamson, R. H., Canellos, G. P., and Sieber, S. M.: Studies on the antitumor activity of gallium nitrate (NSC-15200) and other group IIIa metal salts. Cancer Chemother. Rep. 59: 599-610, 1975.

3. Rabin, H., Adamson, R. H., Neubauer, R. H., Cicmanec, J. L., and Wallen, W. C.: Pilot studies with human interferon in Herpesvirus saimiri - induced lymphoma in Owl monkeys. Cancer Res. 36: 715-719, 1976.

4. Plowman, J., Cysyk, R. L., Adamson, R. H.: The disposition of coralyne sulphoacetate in rodents. Xenobiotica, in press.

5. Cysyk, R. L. and Adamson, R. H.: The pharmacologic disposition of inosine dialdehyde (NSC-118994) in mice, rats, dogs and monkeys. Cancer Chemother. Rep., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03509-13 LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Carcinogenesis, Chemotherapy and Biological Markers in Non-Human Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. H. Adamson	Chief, Lab. of Chem. Pharmacol.	LCP NCI
OTHER:	S. M. Sieber-Fabro	Staff Fellow	LCP NCI
	R. K. McIntire	Senior Investigator	LCB NCI
	P. Correa	Pathologist	Louisiana State University
	D. W. Dalgard	Veterinarian	Hazleton Laboratories

COOPERATING UNITS (if any)

Laboratory of Cell Biology, NCI; Department of Pathology, Louisiana State University, New Orleans, Louisiana; and Hazleton Laboratories, Inc., Falls Church, Virginia

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

Various chemicals are being administered to various species of Old World monkeys to obtain comparative data on the response of primates and rodents to known carcinogens and to materials suspected to be carcinogenic for humans. In addition, it is hoped that biological markers which will aid in the diagnosis of preneoplastic change as well as for frank neoplasia will be developed. Thus far seven chemicals have induced tumors in Old World monkeys; these are diethylnitrosamine, dipropylnitrosamine, 1-nitrosopiperidine, methylnitrosourea, methylazoxymetnanol acetate, aflatoxin B1 and procarbazine. Methylnitrosourea has induced squamous cell carcinomas of the oral cavity; procarbazine has induced acute myelogenous leukemia, lymphoma and hemangiosarcoma while the other carcinogens have all induced primary liver carcinomas. The serum levels of alpha-fetoprotein by radioimmunoassay in monkeys with biopsy-proven liver tumor were scattered over a wide range of 100,000 fold variation which is analogous to the range seen in human hepatocellular carcinoma.

Thus far surgery is the only modality that has consistently reduced the mass of primary liver tumor more than 50% although several chemotherapeutic agents have reduced the tumor mass or the alpha-fetoprotein level by more than 50% in certain monkeys.

Project Description:

Objectives:

This project is designed to obtain comparative data on the response of primates and rodents to known carcinogens and to materials suspected to be carcinogenic for humans and to develop model primate tumor systems predictable for therapy for humans and for development of biological markers. Specifically the objectives are:

To study the effects of compounds, generally of potential carcinogenic activity in newborn primates and to determine if the newborn primate, like the rodent, will be highly susceptible to carcinogenesis.

To obtain data on which to base a comparison of the response of monkeys and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and primates in predicting for man.

To use normal and tumor-bearing primates for studying the pharmacological, toxicological, and chemotherapeutic properties of various anticancer, antiviral, and antimicrobial agents.

To evaluate the long-term effects of anticancer drugs which are being used clinically for long-term remissions and for diffuse collagen disorders.

To develop model non-human primate tumor systems.

To use the various tumors and animals with premalignant changes and with tumors for biochemical and immunological studies.

To develop biological markers which will aid in the diagnosis of preneoplastic changes as well as for existing neoplasia.

Methods Employed:

Usual pharmacologic, biochemical, clinical, anatomical pathologic, cytological, and immunologic techniques as well as breeding and nursing care of infant monkeys and special dietary regimens. The breeding record and health status of the breeding colony is similar to that reported for previous years.

Major Findings:

General Information:

The present colony consisting of 600 animals is comprised of five species: Macaca mulatta, Macaca fascicularis, Cercopithecus aethiops, Galago crassicaudatus, and Aotus trivirgatus. Of these 450 are experimental;

the remaining 150 are adult breeders which supply the newborns for experimental study.

Previous annual reports have contained detailed information concerning the management and maintenance procedures employed, the hematological, biochemical and diagnostic parameters monitored and information concerning the methods utilized in treating the newborns. These procedures have not been significantly altered and are, therefore, not included in this report.

No major disease problems have been encountered during this period.

Compounds Inducing Tumors:

The summary below is for chemicals that have induced tumors in Old World monkeys. Seven of the chemicals employed have induced tumors to date in the three species of Old World monkeys under study - these include 3 nitrosamines: [diethylnitrosamine (DENA), 1-nitrosopiperidine (PIP), and dipropylnitrosamine (DPNA)], methylnitrosourea (MNU), cycasin and its aglycone MAM acetate, aflatoxin B_1, and procarbazine. Methylnitrosourea has induced squamous cell carcinomas of the mouth, esophagus and pharynx and procarbazine has induced acute myelogenous leukemias, hemangiosarcoma and lymphoma while the other carcinogens have all induced primary liver cell carcinomas in Old World monkeys.

The chemicals used which have not been shown to be carcinogenic in Old World monkeys thus far after treating at least 10 monkeys for 5 years include: N-2-fluorenylacetamide, urethene, low density polyethylene plastic, cyclamate, saccharin, and DDT.

The carcinogen studied most extensively is DENA. When given to Old World monkeys intraperitoneally in gradually increasing doses of 10, 20 and 40 mg/kg every 14 days a liver tumor incidence of 100% can be anticipated. The average induction time is 15 months with a range of 6 to 22 months.

To compare the carcinogenic potential of DENA in a prosimian primate (Galago crassicaudatus) with that in Old World monkeys 14 laboratory reared Galagos were utilized. Intraperitoneal injections of DENA were initiated between 1-3 months of age and continued at 2 week intervals until evidence of tumor was detected. The dose levels ranged from 10 to 30 mg/kg. Ten of the Galagos developed mucoepidermoid carcinoma of the nasal cavity. The tumor was first observed as a unilateral nodular swelling which arose from the premaxilla. A mucoid nasal discharge and obstructed nasal breathing were consistently seen. Often the tumor penetrated the cribriform plate producing CNS symptoms. The induction time varied between 14 and 28 months and the total amount of DENA administered was 0.3 to 1.5 grams. Obvious liver pathology was observed at necropsy in the 10 Galagos with nasal tumors but only 2 of these 10 animals also had primary carcinoma of the liver.

The serum levels of AFP using radioimmunoassay in monkeys with biopsy-proven liver tumor were scattered over a wide range of 100,000 fold variation, which is analogous to the range seen in human hepatocellular carcinoma. Monkeys with N-nitrosodiethylamine induced neoplasms had significantly higher serum levels of AFP than those with tumors induced by 1-nitrosopiperidine, cycasin or aflatoxin B_1. Serial determinations of AFP levels demonstrated a tendency for each monkey with liver tumor to plateau after reaching a level characteristic for that tumor.

The half-life of native serum AFP was measured using three different conditions; serum from hepatoma monkeys injected i.v. into adult monkeys, the post-partum decline in neonatal monkeys, and the decline in a hepatoma-bearing monkey following surgical removal of the tumor. Despite many differences among these situations the results tended to corroborate a T 1/2 for AFP of 2.8-3.6 days. In both the neonatal monkeys and post-surgical removal of tumor there may be continued synthesis of AFP, perhaps declining in the neonatal monkeys and increasing in the monkey which may develop recurrent tumor post-surgery. Further work on catabolic rates of AFP where there is a varied total body load and establishment of rates of cellular synthesis will allow the estimation of total body tumor cell number and the changes that take place as a result of various modalities of tumor therapy.

Surgery is the only modality which has consistently reduced the mass of primary liver tumor and serum alpha-fetoprotein by more than 50% although several chemotherapeutic agents have reduced either the tumor mass and/or the AFP level by more than 50% in certain animals. For example, in monkey 815J, three courses of adriamycin at 1 mg/kg over a 6 month period reduced the tumor mass and decreased the serum AFP from 252,000 ng/ml to 10,000 ng/ml. In addition, BCG in two monkeys (925 and 930) has thus far either delayed or prevented the induction of tumor by nitrosamines. Other chemotherapy which has been tried includes poly I·poly C, methotrexate, 6-MP, 5-FU and gallium nitrate with partial responses being seen with methotrexate and gallium.

Several owl monkeys with HVS induced lymphoma-leukemia have been treated with various anticancer agents. The disease responds to steroids, oncovin, cyclophosphamide and cytosine arabinoside. In addition, human interferon (400,000 IU/kg) given daily for 15 days produced a definite decrease in the peripheral white count and markedly reduced swollen peripheral lymph nodes in two of four owl monkeys with HVS induced lymphoma-leukemia. Further trials of human interferon in this disease are planned.

Publications:

1. Correa, P., Dalgard, D. W., and Adamson, R. H.: Olfactory neuroepithelioma in a Cynomolgus monkey (Macaca fascicularis). J. Med. Primatol. 4: 51-61, 1975.

2. Sieber, S. M., and Adamson, R. H.: The clastogenic, mutagenic, teratogenic and carcinogenic effect of various antineoplastic agents. In Pharmacological Basis of Cancer Chemotherapy. Baltimore, Williams and Wilkins Co., 1975, pp. 401-468.

3. Adamson, R. H., McIntire, K. R., Sieber, S. M., Correa, P., and Dalgard, D. W.: Nonhuman primate models for lymphoma, leukemia and other neoplasms. In Ito, Y. and Dutcher, R. M. (Eds.): Comparative Research, Leukemogenesis. Tokyo, Karger, Basel, University of Tokyo Press, 1975, pp. 723-730.

4. Dalgard, D. W., Adamson, R. H., and Vermess, M.: Diaphragmatic herniation of the liver in Macaques demonstrated by intravenous hepatography. Lab. Anim. Sci. 25: 753-756, 1975.

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 CM 06108-07 LCP

PERIOD COVERED

July 1, 1975 to June [illegible]

TITLE OF PROJECT (80 characters or less)

Studies on Mechanism of Action, Mechanisms of Resistance and Anti-Metastatic Activity of Anticancer Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. L. Cysyk	Staff Fellow	LCP NCI
OTHER:	P. E. Gormley	Clinical Associate	LCP NCI
	S. M. Sieber-Fabro	Staff Fellow	LCP NCI
	R. H. Adamson	Chief, Lab. of Chem. Pharmacol.	LCP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacology and Experimental Therapeutics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

Studies designed to uncover molecular aspects of cytotoxicity for several new agents are in progress. The interaction of an acridine derivative (NSC-141549) with nucleic acids was investigated and was found to be selective for A:T base pairs as determined by inhibition of various nucleic acid polymerases using synthetic templates of known composition. This was confirmed by thermal stability studies utilizing these same templates. An analog of uridine, 3-deazauridine (NSC-126849) was found to be phosphorylated in vivo to the corresponding nucleotides by mouse tissues and i.p. implanted L1210 cells. The in vivo phosphorylation rates were greater in L1210 cells than in normal cells and there was an accumulation of 3-deazauridine triphosphate in the L1210 cells. An extension of the pharmacologic disposition studies of inosine dialdehyde (NSC-118994) demonstrated this agent to cross-link plasma proteins and to bind to RBC membranes. These reactions would explain the increased thrombin time and the positive Coomb's reaction exhibited by patients receiving this agent.

Cytosine arabinoside 5'-methylphosphonate was synthesized as an analog of araCMP to circumvent resistance to araC. This compound is a competitive inhibitor of dCMP kinase but is not phosphorylated by this enzyme.

Project Description:

Objectives:

The knowledge of the mechanism of action of anticancer agents and why cells become resistant to them will aid in the design of new chemotherapeutic drugs and will aid in the treatment of neoplastic diseases. Specifically:

To determine the mechanism of action of various anticancer agents.

To determine the mechanism of resistance to various anticancer agents.

To design antitumor agents that will inhibit metastases or tumor growth by affecting blood vessels and fibrin cross-linking.

To rationally design new anticancer agents.

Methods Employed:

Usual biologic, pharmacologic, and biochemical techniques. Among these are tumor transplantation, harvesting of cells, tissue culture techniques, separation and analyses of whole cell DNA, RNA, and protein synthesis. Various enzymes are isolated, purified and the effect of various antitumor agents as inhibitors of these enzymes are studied as well as effects on DNA, RNA and various proteins. Molecular models are also used in drug design.

Major Findings:

Studies on Ara-C-5'-Methylphosphonate (Ara-CMeP)

Ara-CMeP was synthesized as an analog of ara-CMP to circumvent resistance that develops to ara-C as a result of deletion of CdR kinase. Ara-CMeP was inhibitory to leukemias L1210 and P388 in vitro at concentrations 30 fold higher than ara-C. For ara-CMeP to function as an analog of ara-CMP phosphorylation by pyrimidine nucleotide kinases would be necessary. Ara-CMP and Ara-CMeP are equally effective as competitive inhibitors of the phosphorylation of dCMP by dCMP kinase isolated from L1210 cells. In contrast to ara-CMP, which is a substrate for this enzyme, ara-CMeP is not phosphorylated to the nucleoside diphosphate form. This indicates that because of its structural similarity in the pyrimidine portion of the molecule, ara-CMeP can bind as well as ara-CMP to dCMP kinase; however, methylation of the phosphate moiety alters the nucleophilicity of this portion of the molecule such that phosphorylation does not occur. Although these results are interesting in that they expand our knowledge of mechanisms of enzyme catalysis, they also indicate that this type of structural alteration might not lead to agents that would be successful clinically.

Mechanism of Action of NSC-141549 (MeAc)-Methansulfon-m-Anisidide, 4'-(9-Acridinyl-9-Amino) Monohydrochloride

Studies on the mechanism of cytotoxicity of MeAc were continued. MeAc was tested for inhibition of reverse transcriptase activity of avian myeloblastosis virus, DNA polymerase activity of NIH-Swiss mouse embryo, and RNA polymerase activity of E. coli. The viral and cellular enzyme activities were strongly inhibited by MeAc when template-primers of A:T base pairs were used. However, no inhibition of reverse transcriptase activity was obtained with poly rC·oligo dG suggesting that the drug interacts specifically with A:T base pairs. This conclusion was corroborated by studying the inhibition of E. coli RNA polymerase by MeAc with different templates of known composition. Furthermore, the thermal stability of synthetic double-stranded nucleic acid polymers containing A:T base pairs was enhanced by MeAc whereas polymers containing only G:C base pairs exhibited no change in their thermal stability. The thermal stability of calf thymus DNA was only slightly increased by MeAc indicating that the primary base sequence is also an important factor in MeAc intercalation. Several spin-labeled 9-amino acridines were synthesized as nucleic acid probes and their biologic activities determined. These compounds are structurally similar to MeAc but differ in the substituent in the 9-position. Although these compounds will be useful probes for nucleic acids, they were not as cytotoxic or as inhibitory to E. coli RNA polymerase as MeAc.

Mechanism of Action of NSC-126849 (3-Deazauridine)

The in vivo phosphorylation of 3-deazauridine by normal mouse tissues and by i.p. implanted L1210 cells was compared as a function of time after i.p. injection of [^{14}C]-3-deazauridine (300 mg/kg). Two hours after injection, greater than 50% of the radioactivity in L1210 cells was that of 3-deazauridine triphosphate as compared to less than 10% for most normal tissues. In a similar experiment where [^{14}C]-uridine was used in place of 3-deazauridine, there was no accumulation of ^{14}C-UTP. Furthermore, 3-deazauridine is not ultimately incorporated into cellular DNA or RNA; an observation that would explain the accumulation of 3-deazauridine triphosphate. It would appear that 3-deazauridine is cytotoxic as the triphosphate where it could inhibit nucleic acid polymerases or perhaps function as a fraudulent allosteric inhibitor of uridine kinase, an enzyme critical for the control of cellular differentiation and division. We are presently evaluating these possibilities as well as studying the capacity of 3-deazauridine to function as a substrate for the various forms of pyrimidine nucleoside and nucleotide kinases from normal and tumor tissues. The selective toxicity exhibited by this compound might well be related to the tissue distribution of these enzymes.

Mechanism of Action of IdA, NSC-118994 (Inosine Dialdehyde)

Inosine dialdehyde (IdA), an antitumor agent undergoing Phase II clinical trial, was found to cross-link plasma proteins in vitro. This interaction might explain the increased thrombin time reported for patients receiving IdA. The interaction of IdA with proteins can be inhibited by the addition of small molecules containing primary amino groups (e.g., amino acids). These small molecules can also reverse the in vitro cytotoxicity of IdA. The use of such small molecules might prove beneficial in controlling clinical toxicities of IdA. IdA interacts with RBC membranes irreversibly under physiologic conditions. A cross-linking of plasma proteins with RBC membranes in vivo could result in the positive Coomb's reaction exhibited by patients receiving IdA.

Publications:

1. Cysyk, R. L., and Adamson, R. H.: Protein cross-linking properties of the antitumor agent inosine dialdehyde (NSC-118994). Cancer Chemother. Rep., in press.

2. Sinha, B. K., Cysyk, R. L., Millar, D. B., and Chignell, C. F.: Synthesis and biological properties of some spin-labeled 9-amino acridines. J. Medicinal Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06120-05 LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Kinetic Profile on Drug Action In Vivo

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. A. Chabner	Asst. Chief, Lab. of Chem. Pharmacol.	LCP NCI
OTHER:	R. C. Young	Chief, Medicine Branch	MB NCI
	C. E. Myers	Senior Investigator	MB NCI
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	J. Bull	Senior Investigator	CO NCI
	H. M. Pinedo	Visiting Scientist	MB NCI
	R. G. Stoller	Clinical Associate	LCP NCI
	S. A. Jacobs	Clinical Associate	LCP NCI

COOPERATING UNITS (if any)

Medicine Branch, NCI; and Clinical Oncology, NCI

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Biochemical Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2 3/4	1/4

SUMMARY OF WORK (200 words or less - underline keywords)

To develop sensitive and specific methods for assay of methotrexate, active derivatives of 5-fluorouracil, and other antineoplastic agents.

To determine the pharmacokinetics of drugs and the relationship of this data to drug toxicity.

Project Description:

Objectives:

In using any pharmacologic agent, certain critical information is needed in order to design an optimal schedule of drug administration. One must know:

1. The nature and kinetics of distribution and disposition of the agent.

2. Its mechanism of action.

3. The levels of parent drug or active metabolite required for the desired effect on target tissue.

While extensive information is now accumulating with regard to pharmacokinetics and mechanism of action of the common antitumor drugs, little is known about the drug concentrations which produce toxicity and antitumor effects *in vivo*. This important consideration is the object of our research efforts. We have chosen methotrexate as a model agent for study because it is easily measured and active as the parent compound. Secondly, its mechanism of action, the inhibition of dihydrofolate reductase, with consequent block of the conversion of dUMP to dTMP allows one to follow its action *in vivo* by determining the incorporation of ^{3}H-UdR into DNA.

We have also extended our studies to 5-fluorouracil and have made similar correlations between intracellular levels of F-dUMP and inhibition of thymidylate synthesis by 5-FU. These studies will be described in the annual report for the Medicine Branch, NCI, in further detail.

Methotrexate Studies

Methods Employed:

Methotrexate (MTX) levels were measured as previously described by the standard dihydrofolate reductase inhibition assay which allows quantitation of 10^{-9}M or greater MTX, and by a newly devised competitive protein binding assay employing the same enzyme as a binding protein.

Pharmacokinetics of Methotrexate in Man

We have continued our studies of the disposition of methotrexate in patients with carcinoma of the ovary and have added several additional patients to this study. The threshold for inhibition of DNA synthesis in bone marrow continues to be in the range of 1-3 x 10^{-8} in these patients; the phenomenon of preferential accumulation in ascites is being

studied by pharmacokinetic modeling and appears to be the result of limited diffusion capacity of the peritoneal surface in relation to the volume of ascites. MTX binding to ascites and plasma has been investigated and appears to be a constant fraction of total drug, regardless of drug concentration, in the range of 20-50%.

Monitoring of High Dose Methotrexate

We have continued to provide routine monitoring of MTX levels for the clinical services of the Clinical Center. Work thus far has shown the value of monitoring in determining the need for prolonging leucovorin. Plasma levels of MTX at 48 hrs below 5×10^{-7}M have been shown to never be associated with drug toxicity and allow discontinuation of leucovorin rescue with confidence. We have monitored over 125 infusions over the past year, with no significant or life threatening toxicity.

Relationship of Drug Level and Duration of Exposure to Bone Marrow Toxicity

Methotrexate toxicity to bone marrow was studied *in vitro* in semi-solid medium using dialyzed serum to eliminate nucleosides. Toxicity was shown to result from both purine and pyrimidine synthesis inhibition and could be reversed by addition of thymidine and a purine source in a non-competitive fashion. *In vivo* studies using a constant infusion device demonstrated that higher levels of MTX produced more rapidly depletion of bone marrow but the maximum cell kill was the same for all levels of MTX above 10^{-8}M. Recruitment of stem cells to myeloid colony formation during the second 24 hrs of drug infusion took place.

Publications:

1. Stoller, R. G., Jacobs, S., Drake, J., and Chabner, B. A.: The pharmacokinetics of high dose methotrexate. Cancer Chemother. Rep. 6: 19-24, 1975.

2. Chabner, B. A., and Slavik, M.: Prospectives on the clinical use of high dose methotrexate. Cancer Chemother. Rep. 6: 1-2, 1975.

3. Pinedo, H. M., and Chabner, B. A.: The role of drug concentration, duration of exposure, and endogenous metabolites in determining methotrexate cytotoxicity. Cancer Chemother. Rep., in press.

4. Jacobs, S. A., Stoller, R. G., Chabner, B. A., and Johns, D. G.: Dose-dependent metabolism of methotrexate in man and rhesus monkey. Cancer Chemother. Rep., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06121-05 LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Purification and Properties of Carboxypeptidase G1

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. A. Chabner	Asst. Chief, Lab. of Chem. Pharmacol.	LCP NCI
OTHER:	J. R. Bertino	Prof. of Med.	Yale University

COOPERATING UNITS (if any)

Department of Medicine, Yale University, New Haven, Connecticut

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Biochemical Pharmacology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	None

SUMMARY OF WORK (200 words or less - underline keywords)

To investigate potential of this agent for rescue in methotrexate toxicity in man.

Project Description:

Objectives:

1. To investigate the potential prevention of methotrexate toxicity by Carboxypeptidase G_1.

Results:

Preliminary testing of Carboxypeptidase G_1 in man was undertaken in 1973-1974, and indicated the ability of this enzyme to deplete circulating folates, to cleave circulating methotrexate, and demonstrated one instance of hypersensitivity in man. The main effort currently is to obtain sufficient enzyme to investigate its utility as a rescue agent following high dose methotrexate in man. This work is being undertaken by contract at Ft. Dedrick and is covered elsewhere in the DCT annual report.

In addition, the enzyme has proved valuable as a means of identifying α-peptide glutamate groups in folate analogs such as metabolites of methotrexate esters and polyglutamates.

Publications:

None

IMPORTANT STATUS INFORMATION (Actual Project Number) DO NOT use for Updates	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06126-04 LCP

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Purification and Properties of Cytidine Deaminase and Other Enzymes in Pyrimidine Analog Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. A. Chabner	Asst. Chief, Lab. of Chem. Pharmacol.	LCP NCI
OTHERS:	K. R. Hande	Clinical Associate	LCP NCI
	R. G. Stoller	Clinical Associate	LCP NCI
	P. Chang	Senior Investigator	BCRC NCI

COOPERATING UNITS (If any)

Baltimore Cancer Research Center, NCI; Medicine Branch, NCI; and Pediatric Oncology, NCI

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Biochemical Pharmacology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2 1/2	1/2

SUMMARY OF WORK (200 words or less - underline keywords)

Studies have been undertaken to determine the pathways of metabolism of the cytidine analog antineoplastic agents in human leukemic cells and liver. Pertinent enzymes have been purified and characterized, and their influence on the outcome of chemotherapy of AML is being examined.

Project Description:

Objectives:

1. To purify and characterize the enzymes required for cytidine analog metabolism in normal and malignant leukocytes.

2. To elucidate mechanisms of enzyme regulation and the effect of ara-C treatment.

3. To determine the relationship between enzyme levels and response to therapy.

Methods Employed and Major Findings:

Studies of Human Leukemic Cells

The activities of CR deaminase, CdR kinase, dCMP kinase, and dCMP deaminase have been determined in Ficol-enriched populations of myeloblasts from patients with AML and CML-blast crisis. The degradative enzyme CR deaminase is found in high concentration in contrast to the initial activating enzyme, CdR kinase, but the deaminase activity is extremely variable among patients. dCMP kinase is present in very high concentrations (10 fold higher than CR deaminase and 100 fold higher than CdR kinase), and appears to be less variable from patient to patient. dCMP deaminase likewise is found in very high concentrations and is relatively constant in AML patients. Thus CR deaminase seems likely to be the more important determinant of response to ara-C. A prospective study of patients with AML, in whom an enzyme profile is determined prior to treatment with ara-C, is currently underway.

Studies of the Biochemistry of Pyrimidine Enzymes

1. dCMP kinase. Using CEM-human ALL cells as an enzyme source, this enzyme has been purified greater than 600 fold and its biochemical properties (sulfhydryl dependence, substrate specificity, and inhibition by the nucleoside phosphates and product) examined. Of interest was its clear preference for ribonucleotide substrates and its good activity for ara-CMP, ara-UMP and FdUMP, in contrast to its weak activity with dCMP as substrate.

2. Cytidine deaminase. The enzyme from human liver has been purified 1500 fold and its binding to ^{3}H-THU (tetrahydrouridine) examined. The association constant as determined by direct measurement of E-I complex has been compared to the K_i determined by kinetic means and comparable values ($\sim 3 \times 10^{-8}$M) were obtained by both methods. The binding method is being used to determine on and off rate constants, and will be used to develop an assay for THU determination in man.

Uridine-Cytidine Kinase

This enzyme which converts 5-azacytidine to 5-azacytidine PO_4 has been isolated from CEM cells and its affinity for 5-azacytidine found to be extremely weak ($\sim 2 \times 10^{-2}$M). The low enzyme levels in leukemic cells and its weak affinity for U-C kinase suggest that alternative explanations for the activation of 5-azacytidine should be examined.

CMP-dCMP Deaminase

Preliminary experiments indicate the presence of separate enzymes for deamination of dCMP and CMP. We hope to purify and characterize these enzymes and determine their affinity for pertinent cytidine analog substrates.

Publications:

1. Coleman, C. N., Johns, D. G., and Chabner, B. A.: Studies on mechanisms of resistance to cytosine arabinoside: Problems in the determination of related enzyme activities in leukemic cells. Annals N. Y. Acad. Sci. 255: 247-251, 1975.

2. Coleman, C. N., Stoller, R. G., Drake, J. C., and Chabner, B. A.: Deoxycytidine kinase: Properties of the enzyme from human leukemic granulocytes. Blood 45: 791-803, 1975.

3. Stoller, R. G., Coleman, C. N., Hande, K. R., Chang, P. R., and Chabner, B. A.: Pharmacology of cytidine analog metabolism in human leukemia cells. Proc. VII Int. Symp. on Comp. Res. on Leuk. and Related Dis., 1975, in press.

(NIH SCIENTIFIC INFORMATION EXCHANGE
PROJECT NUMBER - DO NOT use this space)

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06127-02 LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (60 characters or less)

Modified Degradation Products of Normal Monkey Liver and Hepatoma tRNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. B. Lakings	Visiting Fellow, IPA	LCP NCI
OTHER:	R. H. Adamson	Chief, Lab. of Chem. Pharmacol.	LCP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	None

SUMMARY OF WORK (200 words or less - underline keywords)

HPLC analytical procedures using anion exchange and anion exclusion chromatography with UV-absorption detection were developed to determine the composition of isolated tRNA from normal and induced-hepatoma monkey livers, the urinary excretion levels of tRNA degradation products, and the methylating ability of individual tRNA methyltransferases. Only minor variations in the tRNA base composition of the normal and hepatoma tRNA's were detected. However, increased urinary excretion of pseudouridine, methylated nucleosides and bases, and β-aminoisobutyric acid were found indicating an increased tRNA turnover rate for the hepatoma tRNA. Methyltransferase activity for the hepatoma monkeys were increased compared to normals; however, large variations in individual methylase activities were found with the hepatoma monkeys having greater thymine, 7-methylguanine and less N^2,N^2-dimethylguanine activity than the normals. These results, along with the increased excretion levels of the hepatoma monkeys, point to aberrant methylation of the tRNA while the tRNA composition indicate increased tRNA turnover.

Project Description:

Objectives:

The project was conducted to evaluate and to clarify further the differences in the formation and degradation of tRNA's between normal and neoplastic states. Comparisons between hepatoma and normal monkeys were made on the following:

1. tRNA methyltransferase activity and capacity.

2. Urinary excretion levels of minor nucleosides, bases and β-aminoisobutyric acid.

3. Base composition of isolated tRNA's.

4. Individual tRNA methyltransferase activities.

Results:

Methyltransferase activity and capacity and urinary excretion levels of selected tRNA degradation products were significantly increased for the tumor bearing monkeys while the tRNA base composition, with the exception of increased thymine, was similar to the normal monkey tRNA composition. Individual methyltransferase activities of the tumor bearing monkeys were increased for certain methylated bases and decreased or unchanged for others.

Publications:

1. Lakings, D. B., Waalkes, T. P., and Mrochek, J. E.: High-resolution liquid chromatographic analysis of methylated purine and pyrimidine bases in transfer RNA. J. Chromatogr. 116: 83-92, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE (DO NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, & WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06133-01 LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cyclic Nucleotide Phosphodiesterase and Its Protein Activator in Neoplastic Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Y. P. Liu	Cancer Expert	LCP NCI
OTHER:	B. A. Chabner	Asst. Chief, Lab. of Chem. Pharmacol.	LCP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Biochemical Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

The cyclic nucleotide phosphodiesterases and the heat stable and non-dialyzable protein activators of phosphodiesterase are present in the following tissue culture cell lines: leukemia CEM, breast cancer MCF-7, human eye cancer #49, #57, Y-79, WERI and the rabbit eye cancer cell lines and also the rabbit eye cancer tissue directly removed from rabbits. All protein activators activate bovine brain cyclic nucleotide phosphodiesterase and the activations are Ca^{++} dependent. In the presence of Ca^{++}, the protein activator of CEM cell was more resistant to trypsin inactivation, presumably because of a Ca^{++} induced conformational change in protein activator to a more helical structure.

PHS-6040

Project Description:

Objectives:

Cyclic AMP is related to cellular morphology, growth and differentiation. Since cyclic nucleotide phosphodiesterase catalyzes its hydrolysis, thereby terminating its action, a study of the protein activator affecting phosphodiesterase activity assumes significance. Specifically the aims are:

1. To determine whether various cancer cells contain phosphodiesterase and protein activator and whether the protein activators stimulate phosphodiesterase from other tissues such as bovine brain.

2. To elucidate the mechanism of activation of phosphodiesterase by the protein activator.

Major Findings:

All the 12,100 x g supernatant fractions analyzed were found capable of stimulating the activator-deficient bovine brain phosphodiesterase. The supernatants also contain cyclic nucleotide phosphodiesterase. These findings suggest that cancer cells contain heat stable and non-dialyzable protein activators and an ubiquitous distribution of cyclic nucleotide phosphodiesterase. Activation of bovine phosphodiesterase by the protein activator required a low concentration of Ca^{++}, and chelation of Ca^{++} by ethylene-glycol bis (β-aminoethyl ether) - N,N'-tetraacetic acid rendered the protein activator inactive. Furthermore, in the absence of added Ca^{++}, the CEM activator activity was abolished in 20 min, whereas in its presence, the activator retained more than 80% of its original activity. Even after 40 min of incubation, some 60% of the activity was still resistant to tryptic treatment. This suggests that Ca^{++} confers stability to the protein activator against tryptic digestion, presumably because protein with folded structure are more resistant to tryptic hydrolysis than those with unfolded ones.

Publications:

1. Cheung, W. Y., Lin, Y. M., Liu, Y. P., and Smoake, J. A.: Regulation of bovine brain cyclic 3',5'-nucleotide phosphodiesterase by its protein activator. In Weiss, B. (Ed.): Cyclic Nucleotides in Disease. Baltimore, University Park Press, 1975, pp. 321-350.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06134-01 LCP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Role of the Lymphatic System in the Absorption and Distribution of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Sieber-Fabro	Staff Fellow	LCP NCI
OTHER:	R. H. Adamson	Chief, Lab. of Chem. Pharmacol.	LCP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacology and Experimental Therapeutics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

The lymphatic absorption and biliary and urinary excretion of 12 radioactively labelled antitumor agents in the rat has been examined. The compounds studied are ^{14}C-CCNU, ^{14}C-methyl-CCNU, ^{14}C-guanazole, ^{14}C-dibromomannitol, ^{14}C-diethylstilbestrol, ^{3}H-prednisolone, ^{3}H-dexamethasone, ^{14}C-6-mercaptopurine, ^{14}C-cytosine arabinoside, ^{3}H-vincristine, ^{14}C-cyclophosphamide and ^{14}C-o,p-DDD. Results to date indicate that only ^{14}C-o,p-DDD is extensively absorbed via the lymphatics. Urinary excretion was the major route of elimination for ^{14}C-guanazole, ^{14}C-dibromomannitol, ^{14}C-6-mercaptopurine, ^{14}C-cyclophosphamide, and ^{14}C-cytosine arabinoside; ^{14}C-diethylstilbestrol, ^{3}H-prednisolone, ^{3}H-dexamethasone and ^{3}H-vincristine were largely excreted via the bile.

PHS-6040

Project Description:

Objectives:

An antitumor agent carried selectively by lymphatic channels would be likely to have therapeutic advantages in treating lymphoid malignancies and tumors which metastasize by lymphatics. One objective of this study, therefore, is to examine the extent to which clinically effective antitumor agents are absorbed and distributed via the lymphatics. Another objective is to define the chemical and/or structural characteristics required for the selective uptake of compounds by lymphatics. In achieving this objective, it would be possible to design antitumor agents for selective lymphatic absorption.

Major Findings:

After the intraduodenal administration of ^{14}C-CCNU or ^{14}C-methyl-CCNU 1.8% and 3.0%, respectively, of the radioactivity was recovered in 24-hour thoracic duct lymph, whereas more than 60% of the radioactivity was excreted in urine. Lymph and urine from rats given an intraperitoneal injection of ^{14}C-guanazole contained 4.1% and 92.0%, respectively, of the administered radioactivity; after an intraduodenal dose of ^{14}C-dibromomannitol, 0.4% of the radioactivity was found in 24-hour lymph and 79.4% in urine. Only small amounts of radioactivity were recovered in lymph from rats treated intraduodenally with ^{14}C-diethylstilbestrol (0.6%), ^{3}H-prednisolone (1.0%), or ^{3}H-dexamethasone (2.3%); for ^{14}C-diethylstilbestrol, 80% of the administered dose was found in bile, and this figure was 55% for prednisolone and 42% for ^{3}H-dexamethasone. ^{14}C-6-mercaptopurine and ^{14}C-cytosine arabinoside were both given intraduodenally, and 0.7% and 1.1% of the ^{14}C-activity, respectively, was recovered in thoracic duct lymph; although neither compound was extensively excreted in bile, 33% (for ^{14}C-6-mercaptopurine) and 63% (for ^{14}C-cytosine arabinoside) of the administered radioactivity was recovered in urine. After an intravenous injection of ^{3}H-vincristine, 0.4% of the radioactivity was found in lymph, 39% in bile and 13% in urine. Eighty percent of the radioactivity administered intraduodenally as ^{14}C-cyclophosphamide was found in the urine; bile and lymph contained 16% and 2% of the ^{14}C-activity, respectively. Following intraduodenal treatment with ^{14}C-o,p-DDD, 21% of the dose was recovered in thoracic duct lymph, 36% in bile and 7% in urine.

Publications:

1. Sieber, S. M.: The lymphatic absorption of p,p'-DDT and some structurally-related compounds in the rat. Pharmacology, in press.

ANNUAL REPORT OF THE LABORATORY OF TUMOR CELL BIOLOGY

NATIONAL INSTITUTES OF HEALTH

NATIONAL CANCER INSTITUTE

July 1, 1975 - - June 30, 1976

This Laboratory is concerned with five areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information in human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell cycle maturation in vitro. (This relates to a major interest of the laboratory: Does leukemia in man result from a block in leukocyte maturation?) (6) Based on new information in the literature and from studies within this Laboratory, new approaches to cancer chemotherapy are evaluated in in vitro and in vivo systems. This is the ultimate goal of the Laboratory.

During the past year a number of findings were reported by investigators from the Laboratory:

1. Demonstration that many cases of human myelogenous leukemia contain reverse transcriptase which is immunologically related to reverse transcriptase from tumorigenic subhuman primate type-C viruses.

2. Reported the isolation of a type-C RNA tumor virus from human myelogenous leukemia cells, after cocultivation and its repeated identification from other clinical samples obtained from the same patient.

3. Demonstrated for the first time efficient transcription of exogenous 70S viral RNA with four purified mammalian viral DNA polymerase (Rauscher murine leukemia, simian sarcoma virus, gibbon ape leukemia virus, and Mason-Pfizer monkey virus).

4. Demonstrated the presence of a high molecular weight (140,000) and low molecular weight (70,000) forms of reverse transcriptase from Rauscher murine leukemia virus.

5. Purified to near homogeneity the glycoprotein 71 and p30 of Rauscher murine leukemia virus and raised antisera against the p30. Preparation of antisera against the gp71 is in progress.

6. Developed chromatography and electrophoretic separation method for mapping tryptic digest of purified reverse transcriptase and RNA tumor virus internal antigens. The procedure involves paper chromatography of tryptic digests in the first dimension and high voltage electrophoresis at pH 3.5 in the second dimension.

7. Purified to near homogeneity DNA polymerase γ, the cellular enzyme which has some template-primer properties similar to viral reverse transcriptase.

8. Demonstrated the presence of two ribonuclease H activities in human leukemic cells.

9. Prepared antisera against the reverse transcriptases of avian myeloblastosis virus, Rauscher murine leukemia virus, gibbon ape leukemia virus, simian sarcoma virus and RD114, and against the cellular DNA polymerase α.

10. Demonstrated the presence of high levels of terminal deoxynucleotidyl transferase in cells of patients with acute lymphocytic leukemia and of some patients with chronic myelogenous leukemia in acute blast phase of the disease. This enzyme may prove to be useful as a diagnostic biological marker for following patients during treatment and in remission.

11. Demonstrated the movement of proviral DNA from the cell cytoplasm to its nucleus in the NRK-SSV/KW 23 system.

12. Developed a chromatographic procedure employing hydroxyapatite, for the isolation and partial purification of type-C RNA tumor viruses from tissue culture fluids.

13. Developed a procedure involving oligo dT cellulose for the isolation and purification of viral 70S RNA from cell free tissue culture fluids.

14. Developed competitive molecular hybridization technique for the measurement of genetic relatedness of nucleic acids.

15. Demonstrated the presence of baboon oncornavirus related sequences in the DNA of uncultured spleen of acute myelogenous leukemia patient (leukocytes of this patient on cocultivation released the putative human virus) by its hybridization to cDNA from the baboon related viral component of the putative human virus and to the RNA from baboon virus.

16. Developed a transfection system using murine cell system as a model for the detection of proviral DNA. In this system it is possible to transfer the genetic information for virus synthesis from viral infected cells to appropriate recipient cells using less than a microgram of DNA per target cell culture.

17. Established a suspension culture system that can support long term growth of some lymphoid populations in the presence of protein factors present in PHA-LyCM. Using this system, it is possible, (1) to grow lymphoid cells containing T cells for a long term in the absence of EBV markers, (2) to grow lymphoid cells that depend on the addition of exogenous factors, and (3) to grow lymphoid cells containing leukemic surface antigens from myelogenous leukemia cells.

18. Development of a procedure for the rapid separation and purification of all known DNA polymerases from mammalian cells.

19. Development of a procedure for the identification of reverse transcriptase in small quantitites of leukemic cells (<1 gm).

20. Demonstration that rifamycin SV derivatives (which were previously reported to inhibit the *in vitro* and *in vivo* activity of RNA tumor viruses after direct incubation) increased the latent period for the development of leukemia in AKR mice when administered to pregnant and to newborn mice.

21. Demonstration that the administration of cordycepin to murine leukemia virus (Rauscher) infected mice inhibited the degree of splenomegaly (50%) but did not inhibit the incidence of leukemia.

22. Demonstration and chracterization of two types of leukocyte colony stimulating factor in medium conditioned by human peripheral blood leukocytes.

23. Demonstration that media conditioned by selected fibroblastic culture cells from human embryos markedly stimulate the growth and differentiation of myelogenous leukemia cells in liquid suspension culture.

24. Demonstration that the endogenous, RNA-directed DNA polymerizing complex in the microsomal fraction of human leukemic cells can be manipulated by gel chromatography and by isopycnic and velocity gradient centrifugation in a similar fashion to RNA tumor virus particles.

25. Demonstration that the genomic RNA of RNA tumor viruses are related to one another in the same manner as the phylogenetic relationship of their respective homologous host cells; this suggests that an RNA tumor virus evolves from the cellular genome.

26. Demonstration that infection of a cell with a RNA tumor virus results in marked alteration of the genome of the merging virus.

27. Partial elucidation of the composition of RNA tumor virus genomic RNA by molecular hybridization and sequence analysis.

28. Demonstration that DNA synthesized by the endogenous DNA polymerase reaction complex of human myelogenous leukemia cells has extensive sequence homology to genomic RNA from primate type-C virus; additionally, there may be sarcoma-specific sequences present in myelogenous leukemia cells.

29. Demonstration that the genomic RNA of selected RNA tumor viruses (subhuman primate; sarcoma-specific) is related to cytoplasmic DNA but not to nuclear DNA from human leukemic cells.

30. Development of microassays for the *in situ* detection of virus-specific nucleic acids (molecular hybridization) or proteins (antibody assays) (in progress).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06117-04 LTCB

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert C. Gallo	Chief, Lab. of Tumor Cell Biology	LTCB	NCI
Others:	Robert E. Gallagher	Senior Surgeon	LTCB	NCI
	Prem S. Sarin	Chemist	LTCB	NCI
	David Gillespie	Microbiologist	LTCB	NCI
	W. Carl Saxinger	Microbiologist	LTCB	NCI
	Anthony Schrecker	Research Chemist	LTCB	NCI
	Jerome Donlon	Staff Associate	LTCB	NCI
	Frank Meyskens	Clinical Associate	LTCB	NCI
	David Strayer	Clinical Associate	LTCB	NCI
	M. Virmani	Visting Fellow	LTCB	NCI
	V. S. Kalyanaraman	Visting Fellow	LTCB	NCI
	Flossie Wong-Staal	Cancer Expert	LTCB	NCI
	Marjorie Guroff	Cancer Expert	LTCB	NCI
	H. Nakazato	Cancer Expert	LTCB	NCI
	Donald Slater	Consultant	LTCB	NCI

COOPERATING UNITS (if any) Richard Adamson, Laboratory of Chemical Pharmacology, National Cancer Institute, George Todaro, Viral Leukemia and Lymphoma Branch, National Cancer Institute; Jeff Schlom, Breast Cancer Segment, National Cancer Institute; Stu Aaronson, Viral Carcinogenesis Branch, National Cancer Institute,

LAB/BRANCH
Laboratory of Tumor Cell Biology

SECTION
Section on Cellular Control Mechanisms and Section on Nucleic Acid Hybridization

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
31	15	16

SUMMARY OF WORK (200 words or less - underline keywords)

This Laboratory is concerned with five areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information to human leukemic cells; (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell cycle characteristics; (5) biochemical and physiological studies on leukocyte maturation in vitro. (This relates to a major interest of the laboratory: Does leukemia in man result from a block in leukocyte maturation?) (6) Based on new information in the literature and from studies within this Laboratory, new approaches to cancer chemotherapy are evaluated in in vitro and in vivo systems. This is the ultimate goal of the Laboratory.

PHS-6040

COOPERATING UNITS (Continued):
Tadao Aoki, Viral Leukemia and Lymphoma Branch, National Cancer Institute; Sol Spiegelman, Columbia University College of Physicians & Surgeons; Robert Ting, Biotech Laboratories; Rolf Neth, University of Hamburg; Natalie Teich, Robin Weiss, Imperial Cancer Research Fund, London, England; Michael Hanna and James Ihle, Frederick Cancer Research Center; Fritz Deinhardt, University of Illinois; Wolfe Prensky, Sloan-Kettering Institute; Ray Gilden, Flow Laboratories; Dani Bolognesi, Duke University; B. Lozzio, University of Tennessee; Ken McCredie, M. D. Anderson Hospital and Tumor Institute; Marcel Baluda, University of California at Los Angeles; Richard Metzgar, Duke University Medical Center.

Project Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

3. To develop new concepts of chemotherapy and apply them to animal model systems as rapidly as possible as new information is derived from basic experimental studies.

These objectives have primarily been pursued by the following approaches:

1. Purification and properties of enzymes of nucleic acid metabolism especially DNA polymerases (DNA-dependent and RNA-dependent) of human normal and leukemic cells and of RNA oncogenic viruses to determine ultimately:

   a. The function, characteristics, and number of DNA polymerases in these cells and viruses.

   b. If RNA-dependent DNA polymerase is present in human leukemic cells and, if so, is it the same as the virion enzyme? Can it copy viral 70S RNA? Can it copy cellular messenger RNA? Does it function in gene amplification during cellular differentiation? Is its activity altered in leukemogenesis? If found, is its activity altered in leukemogenesis? If found, is its activity necessary for maintenance of the neoplastic cell? Can it be used as a marker of neoplastic cells? If it is not necessary for maintenance of the neoplastic state will its continued inhibition prevent some relapses of animals or patients in remission, i.e., does the inciting agent in human leukemia (?virus) remain and produce subsequent transformation?

   c. Is the viral enzyme necessary for viral replication and for initiation of transformation?

Several of the above questions have now been answered.

2. Biochemical studies on the properties of the RNA of type-C viruses and on the overall pathway of replication of these viruses. Purposes:

a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

b. To determine if diagnostic probes can be obtained, i.e., is their structure specific enough that we can use this information to find viral RNA in cells?

c. In understanding the mechanisms involved in integration and expression of viral genes, we can plan approaches to interfere with this expression and then evaluate the overall biological effect of this interference. We particularly wish to know if viral expression is required to maintain the cell in the neoplastic state.

3. Pursuing studies leading to an understanding of the origin of tumor viruses, how they acquire their oncogenic potential, how they interact with cells, and how they are transmitted throughout nature. These studies are primarily carried out with techniques of molecular hybridization.

4. Leukocyte differentiation in vitro. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells was recently set up in our laboratory. Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

5. Growth of leukemic myeloblasts in liquid suspension under the stimulus of a conditioned media factor produced by human embryonic culture cells.

6. Isolation and characterization of type-C virus which was produced by leukemic myeloblasts grown in liquid suspension and the development of biological and biochemical markers for human leukemia.

7. Helping to elucidate the mechanisms involved in the initiation of DNA synthesis in normal and neoplastic cells and the development of potent and selective DNA polymerase inhibitors to be evaluated on fresh human acute leukemic cells and proliferating normal lymphocytes in short term culture.

8. Markers: (a) an immuno-chemical technique for finding reverse transcriptase and other viral macromolecules in intact cells is now being developed. (b) Techniques for detecting viral specific nucleic acids in intact cells are also being developed.

Methods Employed

1. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effects of Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; Blood 37: 282-292, 1971).

2. DNA polymerase activities were purified and characterized as reported (Nature New Biology 240: 67-72, 1972; Proc. Natl. Acad. Sci. 69: 2879-2884, 1972; Proc. Natl. Acad. Sci. 69: 3228-3232, 1972; DNA Synthesis in vitro, Proceedings of the Second Annual Steenbock Symposium, 1972.

3. Viral reverse transcriptase was purified and studied as described (Nature 234: 194-198, 1971: J. Virol. 12: 431-439, 1973).

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells, and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst. 46: 789-795, 1971; Science 165: 400-402, 1969).

5. In vitro leukopoiesis is studied by the soft agar technique developed by Paran and Sachs. In addition human myelogenous leukemic leukocytes are propagated in liquid suspension culture, as recently reported (Science 187: 350, 1975).

6. Induction of type-C virus from "non-producer" cells by iododeoxyuridine is carried out essentially as originally described by Rowe and colleagues. Infectious units focus formation and plaque assays for virus are carried out by conventional techniques.

7. Molecular hybridization studies are carried out by conventional and by newly evolved techniques. These include: (a) filter technique with DNA; (b) filter technique with RNA covalently attached (Proc. Natl. Acad. Sci. 70: 3219-3224, 1973); (c) Cesium sulfate gradient analyses; (d) S_1 nuclease treatment; (d) RNA-DNA hybridization by competition analyses (Methods in Cancer Research, Vol. XI).

8. Tissue culture, virus production, cell viability estimates, cloning of cells are all carried out by standard techniques. Established procedures for titering infectious, leukemic viruses (SX test) and transforming sarcoma viruses (focus formation) are routinely performed. Also, virus neutralization procedures are performed by standard procedures.

9. Virus quantitation, virus specific molecules, metabolism of viral RNA and proteins are studied by conventional techniques.

Major Findings

1. Identification and partial characterization of a conditioned media factor produced by human embryonic culture cells which sustains exponential growth of myeloid leukocytes from patients with myelogenous leukemia in liquid suspension culture.

2. Growth and partial characterization of myeloid leukocytes in liquid suspension of cells from 16 patients with acute or chronic myelogenous leukemia.

3. Identification of a type-C virus produced by one of the cultured human myelogenous leukemia cell lines. Virus isolated from three sublines of a blood sample and later again isolated from a bone marrow sample from the same patient.

4. Demonstration that the virus released from human AML cells is infectious for various homologous and heterologous fibroblastic cell culture lines. Demonstration that this virus can rescue integrated murine sarcoma virus. Adaptation of an in vitro test (XC test) to standardize infectious units of virus activity.

5. Characterization of reverse transcriptase from all the primary isolates and the secondary isolates of the human AML type-C virus. Determination that it is immunologically-related to reverse transcriptase from the two known tumorigenic subhuman primate type-C viruses, gibbon ape leukemia virus and woolly monkey sarcoma virus.

6. Demonstration that the intracellular form of viral-related reverse transcriptase from human AML cells and from type-C virus-producing animal cells may be present in an aggregated form which affects the primer-template and immunological properties of the enzyme.

7. Demonstrated efficient transcription of exogenous 70S viral RNA with four mammalian viral DNA polymerase (Rauscher murine leukemia, simian sarcoma virus, gibbon ape leukemia virus, and Mason-Pfizer monkey virus).

8. Demonstrated the presence of a high molecular weight (140,000) and low molecular weight (70,000) forms of reverse transcriptase from Rauscher murine leukemia virus.

9. Purified to near homogeneity the glycoprotein 71 and p30 of Rauscher murine leukemia virus and raised antisera against the p30. Preparation of antisera against the gp71 is in progress.

10. Developed chromatography and electrophoretic separation method for mapping tryptic digest of purified reverse transcriptase and RNA tumor virus internal antigens. The procedure involves paper chromatography of tryptic digests in the first dimension and high voltage electrophoresis at pH 3.5 in the second dimension.

11. Purified to near homogeneity DNA polymerase γ, the cellular enzyme which has some template-primer properties similar to viral reverse transcriptase.

12. Demonstrated the presence of two ribonuclease H activities in human leukemic cells.

13. Prepared antisera against the reverse transcriptases of avian myeloblastosis virus, Rauscher murine leukemia virus, gibbon ape leukemia virus, simian sarcoma virus and RD114, and against the cellular DNA polymerase α.

14. Demonstrated the presence of high levels of terminal deoxynucleotidyl transferase in cells of patients with acute lymphocytic leukemia and of some patients with chronic myelogenous leukemia in acute blast phase of the disease. This enzyme may prove to be useful as a diagnostic biological marker for the following patients during treatment and in remission.

15. Demonstrated the movement of proviral DNA from the cell cytoplasm to its nucleus in the NRK-SSV/KW 23 system.

16. Developed a chromatographic procedure employing hydroxyapatite, for the isolation and partial purification of type-C RNA tumor viruses from tissue culture fluids.

17. Developed a procedure involving oligo dT cellulose for the isolation and purification of viral 70S RNA from cell free tissue culture fluids.

18. Developed competitive molecular hybridization technique for the measurement of genetic relatedness of nucleic acids.

19. Demonstrated the presence of baboon oncornavirus related sequences in the DNA of uncultured spleen of acute myelogenous leukemia patient (leukocytes of this patient on cocultivation released the putative human virus) by its hybridization to cDNA from the baboon related viral component of the putative human virus and to the DNA from baboon virus.

20. Developed a transfection system using murine cell system as a model for the detection of proviral DNA. In this system it is possible to transfer the genetic information for virus synthesis from viral infected cells to appropriate recipient cells using less than a microgram of DNA per target cell culture.

21. Established a suspension culture system that can support long term growth of some lymphoid populations in the presence of protein factors present in PHA-LyCM. Using this system, it is possible, (1) to grow lymphoid cells containing T cells for a long term in the absence of EBV markers, (2) to grow lymphoid cells that depend on the addition of exogenous factors, and (3) to grow lymphoid cells containing leukemic surface antigens from myelogenous leukemia cells.

22. An attempt was made to isolate and characterize RNA dependent DNA polymerase from 3 sources of murine Xenotropic viruses. NZB mice spleens of NIH swiss mouse radiation lymphoma tissue contained very low levels of viral DNA polymerase. Hartley cell line (34889) was found to be good source for the viral DNA polymerase.

23. Particles with biophysical and biochemical properties of complete RNA-tumor viruses have been cytoplasmically located in some cases of human leukemia. These similarities include comparable buoyant densities and sedimentation velocities in sucrose and relatedness to viral reverse transcriptase, structural protein and nucleic acids.

24. Demonstration that DNA products synthesized by the components of the high density cytoplasmic particles from leukocytes of various leukemic and normal patients contain sequences related to a collection of different RNA tumor viruses; but differences are seen in the extent and pattern of hybridization - differences between normal and leukemic patients and between patients with different forms of the disease.

25. Demonstration that the genomic RNA of RNA tumor viruses are related to one another in the same manner as the phylogenetic relationship of their respective homologous host cells; this suggests that an RNA tumor virus evolves from the host genome.

26. Demonstration that the human virus-like genetic information and the primate leukemia virus information have had similar evolutionary histories by comparison with the above pattern.

27. Demonstration that endogenous (usually nononcogenic) RNA tumor viruses (Class I) are highly related to DNA of the progenitor cell; whereas another group (Class II) thought much less related to DNA from the progenitor cell could still have arisen entirely from the host cell genome.

28. Demonstration that infection of a cell with an RNA tumor virus results in marked alteration of the genome of the emerging virus.

29. Development of an efficient sensitive method for the purification and detection of intracellular reverse transcriptase from $\leq$ 0.1 grams of leukemic cells, using affinity chromatography.

30. Demonstration in a mammalian system that in the endogenous viral DNA synthesis, the primer is a unique species of 4S RNA terminating with 3'-adenosine. Attached to the primer a unique oligodeoxynucleotide sequence of 8-10 residues has been isolated and its sequence partially characterized.

31. Elucidation of the life cycle of RNA tumor viruses following their induction in cells transformed by but not producing virus. In various stages of virus infection (transformation, induction by IdU, inhibition with cordycepin, or stimulation with dexamethasone revertant) the amount of integrated viral DNA, expression of viral RNA and protein, and the production of released virus attain characteristic and discernible levels as measured by nucleic acid hybridization, immunoassay and enzymatic activity.

32. Demonstration that cytoplasmic particulate fraction from a mouse plasma tumor (MOPC-104E) which copurified with intracisternal A particle was able to synthesize a poly A sequence 150 nucleotides long using 35S RNA as a primer.

33. Achievement of partial purification and characterization of CSF from both adherent cells and nonadherent cells of human peripheral blood culture. CSF from the nonadherent cells can only stimulate colony formation of primate marrow cells.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanisms and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Proposed Course

As described above, some projects will terminate and others will continue to be actively pursued.

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ANNUAL REPORT OF THE LABORATORY OF MOLECULAR PHARMACOLOGY

NATIONAL CANCER INSTITUTE

NATIONAL INSTITUTES OF HEALTH

July 1, 1975 - June 30, 1976

The Laboratory of Molecular Pharmacology is engaged in studies of DNA damage, repair, and replication in relation to the effects of chemotherapeutic agents on cells in culture. The major long-range objective is to relate effects on DNA with cytotoxicity, particularly in regard to differential toxicity against different cell types and interactive effects of combinations of 2 agents. Studies during the past year have centered on (1) methodology for measurement of DNA damage, (2) identification of the types of DNA damage caused by alkylating agents and by nitrosoureas, (3) effects on repair of DNA damage, (4) interactive effects between nitrosoureas and ionizing radiation, (5) DNA-protein linkage in chromatin by alkylating agents, (6) the relation between DNA damage and cytotoxicity in the action of nitrosoureas on normal and transformed human cells.

Alkaline elution method for measurement of DNA damage.

This sensitive method for determination of DNA single-strand breakage and crosslinking has been developed in our laboratory over the past 4 years, and its usefulness for studies of DNA damage, repair and replication in mammalian cells has been demonstrated. The detailed biophysical basis for the method, however, is still not fully defined. Towards this end, we examined the size distribution of eluted DNA single-strands by alkaline sedimentation and verified the large size of the affected strands, as well as the previously inferred dependence of elution rate on strand length. These findings help to put the method on a sound foundation. A quantitative theory to account for all of the observed elution phenomena, however, is still elusive.

DNA single-strand breaks in cells treated with BCNU.

Bis-chloroethylnitrosourea (BCNU) produced DNA single-strand breaks observable both by alkaline sedimentation and alkaline elution methods. A detailed study by alkaline sedimentation revealed the dose and time dependences of the formation and apparent repair of breaks in normal human cells (WI-38) and in an SV-40 transformed derivative (VA-13). Although the VA-13 cells were more sensitive to killing by BCNU, the 2 cell lines exhibited no significant differences in the formation and disappearance of DNA breaks. Parallel studies using alkaline elution are under way to determine the possible role of DNA crosslinking.

Interaction between BCNU and X-ray: difference between a normal and a transformed cell line.

We had previously found that nitrosoureas and their isocyanate decomposition products inhibit the rejoining of DNA single-strand breaks in X-irradation

we are now examining the effect of the relative timing of the BCNU and X-ray treatments in normal (WI-38) and transformed (VA-13) human cells. We find that most of the effect of BCNU is expressed prior to exposure to X-ray. A striking difference was noted between the 2 cell lines. In WI-38 cells, BCNU retarded the disappearance of DNA breaks produced by X-ray. But in VA-13 cells, BCNU caused further breakdown of DNA. Although these results were obtained using supra-lethal doses of X-ray, the experiments are now being repeated using the alkaline elution method which will permit measurements in the therapeutic range of X-ray dosage. It will the be possible to correlate effects on DNA with effects on cell viability.

Crosslinking of DNA by chloroethylnitrosoureas and nitrogen mustard.

Although the alkylating potential of chloroethylnitrosoureas has been viewed as monofunctional, we have found that these compounds cause inter-strand crosslinking of DNA, an effect which implies bifunctional action. This effect was observed both in purified DNA by means of an analytical ultracentrifuge method, and in treated cells by means of the alkaline elution method. In both cases, the appearance of crosslinks is delayed, in that crosslinks accumulate with time after removal of drug. We hypothesize that crosslinking results from a 2-step reaction in which the first step is chloroethylation of one DNA site; in the second step, a chloroethylated site reacts with a site on the opposite DNA strand by nucleophilic displacement of chloride. This would result in a covalent bridge between the 2 DNA strands. In support of this mechanism, it was found that most of the crosslinking can occur after removal of free drug and that the reaction is specific for chloroethyl derivatives.

Crosslinking was also observed in cells treated with nitrogen mustard (HN2). In contrast to the case of BCNU, however, crosslinks caused by HN2 were repaired after removal of drug. This repair is effective in permitting cell survival, since low doses of HN2, which produced measurable crosslinking (by alkaline elution), led to nearly complete colony survival of L1210 cells; the crosslinks were completely repaired over a 24 h period.

Other areas of investigation.

Several areas are in early stages of development for possible interest in relation to mechanisms of chemotherapy.

The possibility is being investigated that differential effects of drugs on normal transformed cells in culture may reflect mechanisms that produce chemotherapeutic effects. The differential effect of BCNU in the WI-38/VA-13 cell system was noted above. Further work is needed, however, to determine the significance of this observation.

Studies were begun on drug actions at the level of chromatin. Certain treatments are known to impair the extractability of DNA from cells, perhaps because of induced linkage between DNA and other cell constituents. We are investigating the possibility that alkylating agents may link DNA to identifiable protein species, or different nuclear proteins to each other. Another question being investigated is whether drug action is localized with

respect to the recently discovered fine structure of chromatin.

Studies of mutagenesis in mammalian cells were begun with the objective of relating observable drug effect on DNA to effects on mutagenesis as well on cell lethality. If different DNA lesions are involved in these 2 effects, it may provide a rationale means to select treatment regimens with optimum therapeutic potential and minimum mutagenic insult.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06112-05 LMP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effects of Chemotherapeutic Agents on DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K. W. Kohn	Chief, Lab. of Mol. Pharmacol.	LMP NCI
	L. C. Erickson	Staff Fellow	LMP NCI
	C. B. Thomas	Staff Associate	LMP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Pharmacology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.8	1.7

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of chemotherapeutic agents and radiation on DNA structure and function are being studied in mammalian cell culture by measurements of DNA single-strand breaks and DNA cross-linking. Drug effects in relation to DNA damage and DNA repair are measured by DNA alkaline elution and by ultracentrifugation methods. Agents of current interest include nitrogen mustards, nitrosoureas, bleomycin, and X-ray. Effects on intracellular DNA are being related to survival of colony-forming ability. Interactive effects in combinations of 2 agents are being examined. Studies are planned on the effects of chemotherapeutic agents on DNA strand assembly during DNA replication. Normal and neoplastic cell lines are compared in order to disclose differential cytotoxicity.

Project Description.

Objectives:

(1) To determine the effects of chemotherapeutic agents on DNA structure and function in cells.

(2) To determine the effects of drugs on DNA replication and repair mechanisms.

(3) To relate the above effects with lethality in various cell types.

Methods Employed:

(1) Cell culture of various cell lines in suspension and surface cultures. Cell lines used include normal and virus transformed human embryo cells, human fibroblasts of various origins, and mouse L1210 cells.

(2) Radioactive precursors.

(3) X-ray and UV-irradiation of cells.

(4) Preparative ultracentrifugation: (2) velocity sedimentation in sucrose gradients; (b) equilibrium sedimentation in Cs gradients.

(5) Analytical ultracentrifugation.

(6) Alkaline elution (a new method devised in this laboratory).

Major Findings:

1) Alkaline Elution Analysis of DNA Damage

This method of measuring DNA single strand breaks and cross-links in mammalian cells was recently developed in our laboratory. It is currently the most sensitive available measurement of changes in cell DNA due to treatment with radiation or DNA-damaging drugs, and is capable of quantitating effects of treatments in the therapeutic range of dosage. The method has been successfully applied to studies of DNA damage and repair following X-ray, ultraviolet light, bleomycin, nitrogen mustard, and BCNU.

During the current year, we have obtained further insight into the mechanism of the alkaline elution phenomenon. Basically, the question is what determines the rate at which long DNA strands dissociate from lysed cells and pass through a filter. We had previously determined some of the kinetic dependencies of this process, and had shown that only DNA single-strands are eluted. We have now measured the sizes of these strands eluted from cell lysates after various times in alkali. Samples of eluted DNA were transformed to the top of alkaline sucrose gradients and sedimented to determine molecular weight. The rate of elution was found to be inversely related to molecular

weight. Molecular weights up to 10^9 were discerned in the gradients. It appears however that the large strands, which are over 1 mm in length, may have been broken during elution and transfer to the gradients. A second method we used to determine the molecular weights discriminated by alkaline elution is based on the efficiency of single-strand breakage by X-ray. We determined this efficiency under our experimental conditions to be 37 eV/break. From this we deduced that the apparent target size of DNA in cells is 4×10^9 daltons.

The possible role of non-DNA material in determining DNA elution from cell lysates was investigated by enzymatic treatment of the lysates. When adequate precautions were taken to avoid possible DNase activity, treatment of lysates with proteinase-K or ribonuclease produced little effect on the elution of DNA from cells, whether or not X-irradiated. This indicates that rate of elution is a property of the DNA itself, rather than a function of linkage of DNA to other non-elutable structures.

In cells that were irradiated with ultraviolet light, however, the ability of DNA to be eluted was diminished, and this effect was reversed by treatment of the lysates with proteinase-K. We concluded that ultraviolet light produced linkage of DNA to protein-containing material which prevented elution of DNA single-strands. Similar experiments with nitrogen mustard suggested that some, but not all, of the drug-induced cross-linking involves protein-containing material. The remainder probably consists of DNA inter-strand cross-links.

2) Bleomycin

We had previously found that DNA single-strand breaks resulting from bleomycin treatment of cultured L1210 cells can be measured by alkaline elution. The sensitivity of the measurement was sufficient to detect changes in the DNA produced by treatments which produced only moderate loss of colony forming ability. The magnitude of the bleomycin effect increased with increasing pH of the medium.

We have now correlated the magnitude of the DNA effect with the extent of cell killing (loss of colony forming ability). We find this correlation to be independent of pH, i.e. the effect on DNA correctly predicted the effect on survival of colony forming ability.

We also examined the effects of bleomycin on human fibroblasts, both normal and xeroderma pigmentosum, and found that the production and repair of DNA single-strand breaks was quantitatively similar in the 2 cell types. This indicates that the excision repair system (in which xeroderma cells are deficient) is not involved in the production or repair of DNA breakage which follows bleomycin treatment.

3) Repair of Nitrogen Mustard (HN2)-Induced DNA Cross-links in L1210 Cells

Cross-linking of DNA in cultured L1210 cells was observed with dosage as low as 10^{-7}M HN2 for 0.5 h, a treatment which permitted 75% survival of colony-

forming ability. These cross-links were almost completely repaired 24 h after treatment. Increasing dosage produced exponential decline in survival, and the repair of cross-linking after 24 h was incomplete and dose-dependent. The results suggest that cell survival after HN2 treatment may depend on the amount of DNA cross-linking that remains unrepaired after some period of time.

4) DNA Cross-linking in L1210 Cells Due to Bis-chloroethylnitrosourea (BCNU)

The alkylating ability of BCNU and other chloroethylnitrosoureas has generally been considered to be monofunctional. However, DNA cross-linking has been associated with bifunctionality. Nevertheless, we found that BCNU cross-links DNA. This was demonstrated in treated L1210 cells by alkaline elution, and confirmed with purified DNA by an analytical untracentrifugation method (section 5).

An unusual feature of the cross-linking of DNA by BCNU is that it is delayed. Cross-linking develops over a period of about 6 h after removal of drug (cells were treated for 1 h). Cross-linking was observed after treatment of L1210 cells with doses as low as 16 μM (3 μg/ml) BCNU for 1 h, which yielded 40% survival of colony forming ability.

Thus HN2 and BCNU differ in that cross-linking by HN2 is maximal immediately after treatment and is then repaired, whereas cross-linking after BCNU is delayed and develops after removal of drug. For both drugs, cell survival may depend on the amount of cross-linking present after some period of time.

5) Inter-strand Cross-linking of Purified DNA by Chloroethylnitrosoureas

The ability of chloroethylnitrosoureas to form cross-links between paired DNA strands was verified using purified E. coli DNA. The DNA was reacted with drug for 1 h at pH 7 in a 0.1 M $NaCl$-$NaPO_4$ buffer at 37°. The drug was then removed by alcohol precipitation of the DNA; the DNA was redissolved and incubation was resumed. Cross-linking was determined by transient exposure of the DNA to alkali, followed by analytical ultracentrifugation to equilibrium in a CsCl gradient. The extent of cross-linking was observed to increase for several hours upon incubation of the DNA after drug removal. Thus the delayed appearance of cross-links occurs with purified DNA as it does with intracellular DNA.

Several chloroethylnitrosoureas were observed to produce delayed cross-linking; in the order of decreasing effectiveness they were chloroethylnitrosourea>BCNU>CCNU. The fluoro analogue of CCNU produced much less cross-linking, and methylnitrosourea produced no detectable cross-linking even after high drug concentrations and prolonged incubation periods. The latter observation argues against the possibility that delayed cross-linking proceeds through a depurination mechanism.

We propose that cross-linking occurs by chloroethylation of a nucleophilic site on one DNA strand, followed by a delayed reaction of the chloroethylated site with a second nucleophilic site on the opposite strand. The second reaction

would be driven by release of Cl^- as a leaving group.

6) DNA Single-strand Breaks in Cells Treated with BCNU

Although cross-linking appeared to be the major effect of BCNU on DNA, single-strand breaks were also observed, both by alkaline elution and by alkaline sedimentation. Single-strand breakage became prominent only after several hours of reaction with relatively high drug concentrations. The single-strand breaks appear to be repaired upon incubation after removal of drug. These results were obtained in the WI-38 line of normal human embryo cells and in the VA-13 line of SV-40 transformed derivatives of WI-38. No significant difference was noted between the 2 cell lines in these experiments, despite the observation that VA-13 cells are more sensitive to killing by BCNU than are WI-38 cells. The results suggest that single-strand breakage *per se* is not a major factor in determining the survival of BCNU-treated cells.

7) Effects of the Combination of BCNU and X-ray on DNA in Human Cells

We had previously observed that nitrosoureas and their isocyanate decomposition products inhibit the repair of X-ray-induced single-strand breaks. In these experiments, drug was added immediately after X-ray, and the isocyanates were more effective than the parent nitrosoureas. We now find that addition of BCNU 1 h before X-ray produces a much greater inhibition of repair than was previously seen. A difference was noted between the normal WI-38 line and the SV-40 virus transformed VA-13 line: whereas DNA in X-irradiated WI-38 tends to rejoin slowly in the presence of BCNU, in the case of VA-13 cells DNA becomes progressively degraded. This difference may be significant, since the 2 cell lines show no difference in DNA effects produced by either BCNU or X-ray as single agents.

These experiments were carried out by alkaline sedimentation using supra-lethal doses of X-ray. The experiments are now being repeated by alkaline elution, which permits measurements in the therapeutic range of X-ray dosage.

Significance to Biomedical Research and the Program of the Institute

The DNA effects measured in this work may be determinants of cell viability following treatment with DNA-damaging agents. If so, it should be possible to use this type of measurement to determine relative sensitivities of different cell types to such agents, and to determine synergism in the action of combinations of agents.

Proposed Course:

1) Determine applicability of alkaline elution method to tissues.

2) Determine what type of DNA damage (if any) correlate with survival of drug-treated cells, using various cell types, including resistant lines. Drugs to be examined: alkylating agents, nitrosoureas, bleomycin, platinum complexes, triazenoimidazoles.

3) Studies of 2-agent combinations. Determine the effect of one agent on DNA damage and repair caused by a second agent. Complete the current study of the combination BCNU plus X-ray, and look for other interactions that produce differential effects in normal and neoplastic cell lines.

4) Examine the strand-joining phase of DNA repair synthesis in cells treated with DNA damaging drugs.

5) Look for perturbations produced by anti-metabolites on the process of replicative DNA strand assembly.

Publications:

1. Friedman, C.A., Kohn, K.W., and Erickson, L.C.: DNA chain growth during replication of asynchronous L1210 cells. Alkaline sedimentation studies. Biochemistry 14, 4018-4023, 1975.

2. Fornace, A.J., Jr., Kohn, K.W., and Kann, H.E., Jr.: DNA single-strand breaks during repair of UV damage in human fibroblasts and abnormalities of repair in xeroderma pigmentosum. Proc. Natl. Acad. Sci., U.S. 73, 39-43, 1976.

3. Fornace, A.J., Jr., and Kohn, K.W.: DNA-protein cross-linking by ultraviolet radiation in normal human and xeroderma pigmentosum fibroblasts. Biochim. Biophys. Acta, in press.

4. Woolley, P.V., III, Dion, R.L., Kohn, K.W., and Bono, V.H.: Binding of 1-(2-chloroethyl)-3-(cyclohexyl)-1-nitrosourea to L1210 cell nuclear protein. Cancer Res. 36, 1470-1474, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (DO NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 6132-01 LMP

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biological Effects of Chemotherapeutic Agents on Cultured Human and Rodent Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. O. Bradley	Sr. Staff Fellow	LMP NCI
Other:	K. W. Kohn	Chief, Lab. of Mol. Pharmacol.	LMP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Pharmacology

SECTION

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.2	1.3

SUMMARY OF WORK (200 words or less - underline keywords)

We are utilizing an in vitro system of normal and transformed human cells for testing various chemotherapeutic stratagems and for studying certain aspects of differential growth control. In our system one combination of drugs will selectively kill transformed human cells while allowing normal cells to survive. We plan to pursue the basis of selective toxicity in vitro and hope to suggest rationales for combination chemotherapy based on our findings. A second area of interest is the biological manifestations of DNA damage as measured by mutation analysis. We have found that BCNU and MNU are mutagenic in V79 CHL cells. Mutagenicity and DNA strand-breakage seem to be correlated in this system.

PHS-6040
(12-75)

Project Description:

Objectives:

We will continue to develop our in vitro system of normal and malignant human cells. We will examine whether various agents have selective toxicity for either normal or malignant human cells in both growth and cloning assays. Attempts will be made to enhance toxicity for the transformed cells while reducing toxicity for the normal cell. Combinations of drugs, heat, light, and X-ray will be used in there attempts to enhance selective toxicity.

A second major objective is to further analyze the relationship in mammalian cells between mutagenicity, toxicity and DNA breakage after treatment with various nitrosoureas alone and in combination with X-rays. We will expand our mutagenicity assay to include the $Na\text{-}K^+$ ATPase markers for ouabain resistance.

We will continue studies of protein degradation in normal and transformed human cells. One question is whether protein-reactive drugs, such as BCNU, alter the rate of protein degradation differentially in normal and transformed cells. Another question is whether toxic drug concentrations induce a generalized lysosomal breakdown followed by accelerated degradation of cellular proteins.

Methods:

(1) Cloning and monolayer culture of WI-38, VA-13, CHO, and CHL cells.

(2) Mutation assays using the CHL cell and 6-thioguanine resistance.

(3) Replica plating of mammalian cells.

(4) Measurements of DNA repair using alkaline elution and alkaline sucrose gradients.

(5) Inverted phase photomicroscopy

(6) Incorporation studies of radioactive drugs and DNA, RNA, and protein precursors.

(7) Pulse-chase and approach to equilibrium measurements of protein degradation in normal and transformed human cells.

Major Findings:

(1) Normal human cells in vitro can be protected from killing by hydroxyurea if they are kept non-proliferating during drug treatment. This protection can be provided by either density-dependent inhibition of growth or by the drug aminonucleoside of puromycin. In contrast, the transformed human cells

are killed by the same protocols because they continue to cycle. These results suggest that it should be valuable to find agents that can reversibly inhibit cycling of normal human cells.

(2) Mixed cultures of normal and transformed human cells can be used to monitor relative survival after various drug regimens.

(3) BCNU added to mixed cultures of normal and transformed human cells provides an initial selective kill of transformed cells. Not all transformed cells are killed and they eventually overgrow the normal cells.

(4) We have examined the regulation of protein degradation in normal and transformed human cells in culture during various growth regulatory states. We conclude from our work that the rate of protein degradation is independent of growth rate in normal and transformed human cells. However, the rate of degradation is closely coupled to medium alterations. This coupling may represent either a coordinated cellular regulatory mechanism or a response to cellular stress caused by sub-optimal conditions.

(5) BCNU is a weak mutagen in hamster cells, while MNU is a strong mutagen. However, BCNU is much more toxic to CHL cells than is MNU.

(6) There is a positive correlation between the amount of DNA strand breakage after BCNU treatment and the drug induced mutant frequency.

(7) We have examined the repair of X-ray induced single-strand DNA breaks in progeric and senescent human fibroblasts. Our methods included DNA alkaline elution and alkaline sedimentation. All senescent and progeria fibroblasts that we have tested repair X-ray damage as efficiently as do normal fibroblasts. The amount of crosslinked DNA is not increased during senescence in these cells. All of our evidence is contrary to some of the major theories of aging.

Proposed Course:

(1) To expand our studies on selective toxicity of transformed human cells in culture. We will examine combinations of drugs and physical treatments, such as X-ray and heat, with various schedules in order to suggest rationales for treatment that might protect normally proliferating cells and more efficiently kill malignant cells.

(2) To attempt to correlate selective toxicity in our human system with DNA damage and repair.

(3) To determine the effects of various agents, such as BCNU, on protein degradation in WI-38 and VA-13.

(4) To further characterize the mutagenicity of other nitrosoureas and to compare relative mutagenicity and toxicity.

(5) To attempt to correlate mutagenicity and toxicity with damage to DNA as measured by alkaline sedimentation and elution methods.

(6) To develop replica plating methods in mammalian cells for use in detecting drug sensitive mutants.

Publications:

1. Bradley, M.O., Erickson, L.C., and Kohn, K.W.: Normal DNA Strand Rejoining and Absence of DNA Crosslinking in Progeroid and Aging Human Cells. Mutation Res., in press.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1975 - June 30, 1976

The Clinical Oncology Program comprises of five intramural Branches concerned with the treatment and investigation of patients with cancer, and incorporates the disciplines of surgery, radiation, medical oncology, and pediatric oncology. Primary emphasis is placed on combined modality treatment of malignancy utilizing surgery, adjuvant chemotherapy, radiotherapy, and immunotherapy for patients with localized or regional disease, and radiation, combination chemotherapy and immunotherapy in selected patients with more advanced cancer. The treatment programs are organ-site oriented, and involve a wide variety of malignant conditions, including leukemias, malignant lymphomas, cancer of the breast, ovary, pancreas, lung, colon, stomach, as well as malignant melanoma, sarcomas of bone and soft tissue, and a variety of childhood malignancies. Treatment protocols are regularly reviewed by the newly-constituted NCI Clinical Research Committee and are coordinated through the office of the Associate Director for Clinical Oncology, Clinical Director by weekly meetings of the Clinical Branch Chiefs. Statistical support for clinical trial design, data compilation and analysis are provided by the Biostatistics and Data Management Section, under the purview of the Associate Director for Clinical Oncology, Clinical Director's office.

Two organizational changes instituted this year have facilitated patient recruitment and followup. The Admitting Office now functions as triage of patient referrals to the appropriate Branch, and the position of admitting officer has been abolished. The Cancer Registry has been incorporated into the Biostatistics and Data Management Section, and will maintain surveillance on admissions and deaths and assist in patient followup when necessary.

Laboratory and clinical research related to the clinical programs are undertaken in each Branch. These range widely in scope, from bedside studies of cancer diagnosis, staging, and drug pharmacology, to basic molecular biology of oncogenic viruses, tumor immunology, and cellular kinetics. These investigations are undertaken by senior staff physicians who have developed a wide variety of laboratory skills; they form a vital link in the provision of both excellent medical patient care and the performance of relevant laboratory studies. There is close cooperation and collaboration among the clinical Branches, as well as with many other Departments and Institutes within the NIH.

Four clinical branches, Medicine, Pediatric Oncology, Surgery, and the NCI-VA Medical Oncology Branch have undergone major organizational changes in the past year, reflecting new leadership and changing emphasis in clinical investigation. Dr. Robert C. Young, Chief of the Medicine Branch, replaced Dr. Vincent T. DeVita. This Branch incorporated the Cytogenetic Oncology Section, the bone marrow culture investigations, and the former Medical Breast Cancer Service. Dr. Arthur Levine, newly appointed Chief of the Pediatric Oncology Branch, has selected additional senior staff and reorganized program goals. The new Chief of the Surgery Branch, Dr. Steven Rosenberg, has introduced studies emphasizing adjuvant chemoimmunotherapy, and has recruited new staff to head laboratories in tumor immunology and surgical metabolism. Dr. John Minna, newly appointed Chief of the NCI-VA Medical Oncology Branch, has also attracted new staff and has instituted a diversity of clinical protocols. As with all investigative programs undergoing change of leadership and reorganization, results of new projects are not yet available. This report will summarize some of the major new findings and describe important new or ongoing projects.

The Clinical Oncology Program has developed a nationally recognized Oncology Fellowship, accredited for eligibility by the Medicine, Pediatric, and Radiation Boards. Clinical Associates (fellows) are assigned to the Branch, and spend a full year with major clinical responsibilities, with a three-month elective in another area; the second year provides experience in the laboratory, with maintenance of some clinical responsibilities. Selected candidates may remain for a third year of continued investigation. In addition to the post-graduate program, clinical electives within the program are available for medical students.

Program Accomplishments

Office of the Associate Director for Clinical Oncology and Clinical Director

This office is responsible for administrative support for the Clinical Oncology Program, and coordinates personnel, equipment, and space allocation. In addition, the dual responsibility of the Associate Director for Clinical Oncology as Clinical Director extends to all clinical activities of the NCI, including protocol review, patient recruitment, patient care, bed allocation, and coordination of education and training of students, nurses, and Clinical Associates. A critical function is the facilitation of interdisciplinary collaborative research between the clinical Branches, the laboratory, and the related programs within the Institute. The Clinical Director represents the entire NCI clinical program in the newly constituted NIH Clinical Center Medical Board. Extramural responsibilities extend to coordination of the clinical and resource contract efforts, organization of relevant "state of the art" seminars and conferences, and clinical consultation for a large number of inquiries from patients and physicians around the country.

Biostatistics and Data Management Section

This section provides invaluable program support in assisting with trial design, data collection (with computerized storage and retrieval systems) data analysis, and research in relevant statistical methodology and mathematical models. A cell kinetics model to improve chemotherapeutic strategy is being tested currently in an animal tumor system.

Medicine Branch

A ten-year followup on a series of 194 patients with advanced Hodgkin's disease treated with MOPP chemotherapy has shown that Hodgkin's disease is cured with drug treatment and: 1) all relapses occurred within 42 months of completion of therapy; 2) 66% of patients with complete responses never relapsed; and 3) 82% of the 155 patients achieving complete remissions are alive at 5 years and 72% at 10 years. Apart from response, continuous disease-free survival was adversely influenced by "B" symptoms, advanced stage, and histological factors.

An impressive review of staging criteria and procedures has demonstrated that non-Hodgkin's lymphomas with diffuse histological patterns should be managed as disseminated disease; exploratory laparotomy added little to less invasive staging procedures. A recently completed trial of C-MOPP versus BACOP chemotherapy in diffuse histiocytic lymphoma revealed equivalent response rates (overall 46% complete responses), remission duration and survival. Eighty per cent of patients with complete responses are alive and free of disease at 3 years. A trial comparing total body irradiation and CVP therapy in lymphocytic lymphoma also revealed equivalent response rates.

Peritoneoscopy has become an essential staging tool in patients with ovarian carcinoma, and is of considerable help in assessing therapy. A recently completed trial of 56 patients with advanced ovarian cancer tested melphalan or combination chemotherapy (hexamethylmelamine, cytoxan, adriamycin, fluorouracil). Response rates were better with the combination (61% vs 79%) and this is reflected in improved survival.

The chemical pharmacology of high dose methotrexate with leucovorin rescue has been studied extensively. Alkalinization of the urine and serial methotrexate levels are important determinants in monitoring toxicity. Serum creatinine, on the other hand, was not a useful guide in predicting toxicity. Clinical pharmacological studies have provided considerable insight into methotrexate activity, toxicity, and mechanisms of drug resistance, and complement collaborative clinical trials in patients with osteosarcoma, head and neck cancer and other malignancies.

One investigative area of particular importance is the study of hormone receptors in breast cancer. The ability to culture human breast cancer cells which vary in hormone receptors and hormone dependency open new avenues of research into mechanism of hormone action in this disease, as well as opportunities to study the genetics of this phenomenon using cell hybrids. A sensitive radioimmunoassay for casein will permit study of hormone-dependent protein synthesis, and may lead to a tumor marker of clinical utility.

A recent review of cytogenetic abnormalities in acute lymphocytic leukemia has revealed aneuploidy in 42% of cases. Long survival was significantly associated with the elimination of the aneuploid line from the marrow.

Experiments with fractionated lymphocytes have revealed a population of "null" cells which possess pleuripotent "stem cell" properties in a in vivo murine assay system.

NCI-VA Medical Oncology Branch

Major emphasis in the past year has been in the staging and aggressive treatment of small cell carcinoma of the lung. A recent trial revealed a 90% overall response rate using combination chemotherapy (cytoxan, methotrexate, CCNU) and a current study emphasizes intensive multi-drug cyclic therapy which has yielded thus far a 60% complete response rate. As some patients have enjoyed remissions in excess of 2 years, this disease, even in advanced stages, appears potentially curable. While a laminar-air-flow facility did not afford a therapeutic advantage, it is now clear that intensive chemotherapy will induce complete remissions, and the goal of therapy will be to prevent relapse.

New trials in gastrointestinal cancer (stomach, colon) have begun in collaboration with Georgetown University Medical School, and protocols for the treatment of multiple myeloma, pancreatic cancer, prostatic cancer, and liver cancer are being designed. These studies will complement other clinical trials in the Institute, and will emphasize intensive combination chemotherapy.

Laboratory studies in the NCI-VA center currently on genetic aspects of malignancy employing somatic cell hybrids. Using these techniques, it has been possible to begin "mapping" human chromosome genes, especially those involved in the regulation of DNA polymerases and those responsible for carcinogen metabolism.

Pediatric Oncology Branch

Seventy patients have entered a recently completed trial utilizing POMP induction, intensive consolidation, prophylactic cranial irradiation with intrathecal chemotherapy, and chemo-immunotherapy during maintenance. A 90% complete remission rate was observed, with 50% disease-free survival at 3 years. The trial demonstrated no additional clinical benefit of BCG immunotherapy during remission. Analysis of these results revealed two distinct prognostic groups, the major determinants of which were ease of initial remission induction and initial WBC. A new protocol addresses the role of aggressive chemoimmunotherapy of the poor prognosis group, while affording optimal therapy for patients at "standard" risk. In addition, immunotherapy with BCG and Raji cells (which elicit a cytotoxic antibody which kills acute leukemia blasts in vitro) is being explored in patients who relapse. The simultaneous administration of these vaccines yields an optimal antibody response, but clinical benefit has not yet been demonstrated. Finally, a recently completed study using an Ommaya resevoir, reveals that intrathecal methotrexate given in a "concentration X time" schedule is equally efficacious and less toxic than "conventional" intrathecal dosage in the management of meningeal leukemia.

One of the objectives of chemotherapy protocols in childhood solid tumors is the incremental advantage of maximal tumor cell kill utilizing "ablative" drug schedules with optimal hematological and microbiologic supportive care. Three clinical trials validate this approach with encouraging results. One of three patients with refractory rhabdomyosarcoma has achieved a 9+ month remission following intensive therapy with a bone marrow autograft. Similarly, two of five patients with relapsing drug-resistant Burkitt's lymphoma (a situation known to be inevitably fatal) have enjoyed 6+ and 14+ month remissions after ablative chemotherapy and bone marrow autografts. Four patients with relapsing neuroblastoma have responded to ablative chemotherapy; although three have died of infectious complications, one was completely free of tumor at autopsy. These results are exceptionally encouraging when one considers the heretofore hopeless situation of drug-resistant relapse. As more is learned of drug scheduling, extramedullary toxicity, and improved supportive care, this approach may well be advanced to first-line therapy in a high risk clinical setting.

A clinical trial testing the utility of short-term granulocyte transfusions in leukopenic patients with gram-negative bacteremia is nearing completion. Twenty-seven such patients entered this randomized trial, and the results reveal that granulocyte transfusion benefited only those who failed to recover autologous leukocytes within a (median) 8-day period. This important result provides clinical criteria for granulocyte transfusion where such resources are limited. Cells collected by the IBM cell separator were equally effective as cells derived from "leukopak" procedures; although the latter gave a higher yield of granulocytes, these cells were physiologically impaired and produced a high frequency of toxic reactions.

Laboratory studies in this Branch have focused on immune functions in leukemia, and on the molecular biology of viral oncogenesis. Major advances in immunology include: 1) the finding that "T" lymphoblasts fail to stimulate allogeneic cells in mixed leukocyte culture; and 2) "T" lymphoblasts have low or absent steroid receptors, compared to "null" lymphoblasts. These findings may indicate that the poor prognosis in such patients is related to both immune and pharmacological phenomena. Major findings in the area of viral oncogenesis include the collaborative discovery of genetic determinants for murine leukemia virus production, and the ability to purify large quantities of Epstein-Barr virus (EBV) DNA for hybridization analyses. Two of 14 patients with American Burkitt's lymphoma were shown to harbor EBV genomes in their tumor.

Radiation Oncology Branch

Continued studies in the use of total body irradiation in patients with chronic lymphocytic leukemia, with the recent addition of chemotherapy, reveal considerable promise in the long term management and survival of patients with symptomatic disease. Surveillance of patients with Hodgkin's disease treated between 1965-69 reveals survival advantage in patients with extended field and prophylactic radiation of non-involved areas. The complete response data are particularly compelling in patients with early stage nodular sclerosing Hodgkin's disease, where 90% of patients are disease free at 5 years following radiotherapy as the sole treatment modality. Aggressive

radiotherapy combined with chemotherapy has vastly improved the response rates and survival in patients with metastatic Ewing's sarcoma. Encouraged by these results, a similar approach in patients with small cell carcinoma of the lung has yielded 20 of 21 complete remissions, some now past their first anniversary free of tumor.

The development of computerized axial tomography has greatly facilitated clinical staging of solid tumors. The Radiation Oncology Branch has recently purchased a total body EMI scanner which will permit better localization of visceral and soft tissue tumors for radiation treatment ports and disease surveillance.

Surgery Branch

While preliminary, two newly devised studies in the Surgery Branch address critical questions in the management of patients with primary malignant melanoma and soft tissue sarcoma. Modeled after Zbar and Rapp's guinea pig system, patients with primary cutaneous melanoma with level IV or IV invasion are randomized to conventional wide excision or to receive intralesional BCG followed in two weeks by complete excision of the primary site. Twelve patients have entered the study with no recurrences in either group to date. Of considerable importance is the observation of complete eradiation of residual primary melanoma in the BCG group. This therapy is well tolerated, though it will take several years to fully evaluate its effect on prevention of local and distant recurrence.

A randomized trial of the role of radical surgery in the management of patients with primary soft tissue sarcoma is underway, with over 20 patients entered on study. This trial will determine the role of post-operative radiation therapy plus chemoimmunotherapy in patients who have a simple excision of the primary tumor, versus the adjuvant effect of chemotherapy in patients undergoing radical surgery for the primary tumor.

Laboratory studies in the Surgery Branch center on tumor immunology and metabolic effects on tumor growth. A recent advance with potential clinical applicability is the observation of the restorative effect of thymosin on *in vitro* T cell number and function in patients with malignancy undergoing radiation therapy. Thymosin is now under clinical study in patients with lung cancer treated with mediastinal radiation. Newly initiated clinical and laboratory studies examine the inceraction of nutrition on tumor growth and tumor-associated immunity. This effort complements a clinical hyperalimentation program as a means of nutritional support in cancer patients.

UNIT: CLINICAL SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06308-05 COP

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biostatistics and Data Management Section

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	M. Kruse	Medical Record Librarian	COP, DCT, NCI

COOPERATING UNITS (if any)
Medicine Branch, COP, DCT, NCI; Pediatric Oncology Branch, COP, DCT, NCI; NCI-VA Medical Oncology Branch, COP, DCT, NCI; Radiation Oncology Branch, COP, DCT, NCI; Surgery Branch, COP, DCT, NCI; and Immunology Branch, DCBD, NCI

LAB/BRANCH

SECTION
Biostatistics and Data Management Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.75	2.75	0

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of the Biostatistics and Data Management Section is the utilization of statistical methodology and computer technology to maximize knowledge obtained from clinical trials and other research carried out within and on contract to the clinical programs of the Division of Cancer Treatment. The Section collaborates in the design and analysis of clinical trials and other experiments, develops data collection procedures and related computer systems, and maintains computer files of collected data. The section also conducts biometric research in support of the above goals. A list of projects in which the section is actively involved follows.

1. Participation in the design and conduct of clinical research.

The section provides statistical consultation for the design of therapeutic clinical trials and related laboratory research within the Division of Cancer Treatment. The section also develops and maintains computer systems of detailed patient data, and serves as the statistical center for design, randomization, data collection and analysis for intramural clinical trials and for collaborative clinical trials in ovarian cancer and metastatic breast cancer.

2. Statistical Analysis

The section provides statistical consultation and collaboration in the analysis of clinical studies. Collaborations this year have included: (a) comparison of CAF vs CMF in the treatment of metastatic breast cancer; (b) evaluation of a combination of Dibromodulcitol and Adriamycin in the treatment of metastatic breast cancer; (c) evaluation of the role of the efficacy of receptor assays in predicting response to hormonal treatment in patients with breast cancer; (d) evaluation of prognostic factors in patients with diffuse histiocytic lymphoma; (e) prognostic factors and long-term follow-up in the treatment of Hodgkin's disease; (f) prognostic factors in the surgical treatment of melanoma; (g) prognostic factors in the surgical treatment of bony and soft-tissue sarcomas; (h) evaluation of the in vitro effect of thymosin on T-lymphocyte level; (i) evaluation of prognostic factors and maintenance chemotherapy in the treatment of ALL; (j) evaluation of chemotherapy and RAJI immunotherapy in advanced ALL; (k) evaluation of intrathecal methotrexate therapy via an Omaya resevoir in the treatment of CNS leukemia; (l) evaluation of regimens for the prophylaxis of CNS leukemia in patients with ALL; (m) correlation of quantitative changes in B or T lymphocyte populations to immunological responsiveness in patients with Burkitt's lymphoma; (n) evaluation of combination chemotherapy in the treatment of advanced lung cancer; (o) comparison of CAF, CFP, and CA in the treatment of metastatic breast cancer; (p) comparison of adrenalectomy to combination chemotherapy in the treatment of metastatic breast cancer; (q) evaluation of the effects of chemotherapy, total body radiotherapy, and bone marrow transplantation on the immunological status of tumor-bearing and normal dogs.

3. The section serves as a general computer resource for the clinical programs of the DCT. Computer systems are developed, and a variety of computer files are maintained for serum bank inventories, immunological monitoring data, selection of platelet and granulocyte donors, and for evaluation of the effectiveness of blood product collections and transfusions.

4. The section maintains a tumor registry and occupancy statistics for patients admitted to the Clinical Center, and performs follow-up for selected patients. Inquiries from outside tumor registries are handled by the section.

5. Modeling and Biometric Research

Development of a mathematical model for predicting effective schedules and doses and effective ways of combining drugs and modalities in the treatment of solid tumors. A promising model has been developed which does not require detailed knowledge of cell kinetic parameters. Experiments for testing this model are underway in the B-16 melanoma, 13762 mammary adenocarcinoma, and the Martin spontaneous mammary carcinoma.

Investigation of sequential termination and treatment assignment rules for the development of clinical trials which require fewer total patients and assign fewer patients the inferior treatment than is accomplished by traditional designs.

Investigation of the effects of stratified randomization on the power and significance level of statistical tests.

Development of Bayesian methods for the escalation of drug dosages in Phase I studies.

Development of methods of estimating sample sizes required for randomized chronic disease studies utilizing prognostic factor information.

Publications:

1. Simon, R., Weiss, G. H. and Hoel, D. G.: Sequential analysis of binomial clinical trials. Biometrika 62: 195-200, 1975.

2. Simon, R., Weiss, G. H. and Hoel, D. G.: A class of sampling schemes for selecting the better of two binomial populations. J. Statistical Computation and Simulation 4: 37-47, 1975.

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5. Looney, W. B., Mayo, A. A., Kovacs, C. J., Hopkins, H. A., Simon, R. and Morris, H. P.: Solid tumor models for the assessment of different treatment modalities: II. Rapid, intermediate, and slow growing transplantable rat hepatomas. Life Sciences 18: 377-390, 1976.

6. Primack, A., Vogel, C. L., Kyalwazi, S. K., Ziegler, J. L. and Simon, R.: A staging system for hepatocellular carcinoma: Prognostic factors in Ugandan patients. Cancer 35: 1357-1364, 1975.

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8. Leventhal, B., Levine, A., Graw, R., Simon, R., Freireich, E. J. and Henderson, E.: Long term second remissions in acute lymphatic leukemia. Cancer 35: 1136-1140, 1975.

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11. Hoel, D. G., Weiss, G. H. and Simon, R.: Sequential tests for composite hypotheses with two binomial populations. J. Royal Statistical Soc. (in press).

12. Byar, D. P., Simon, R., Friedewald, W. T., Schlesselman, J. J., DeMets, D. L., Ellenberg, J. H., Gail, M. H. and Ware, J. H.: Randomized clinical trials: Perspectives on some recent ideas. New Engl. J. Med. (in press).

13. Margileth, D. A., Oster, M. W., Simon, R. and Leventhal, B. G.: Prognosis and lymphoblast size in acute lymphatic leukemia. Brit. J. Hematol. (in press).

14. Leventhal, B. G., Poplack, D. G., Johnson, G. E., Simon, R., Bowles, C. and Steinberg, S.: The effect of chemotherapy and immunotherapy on the response to mitogens in acute lymphatic leukemia. In Oppenheim, J. (Ed.): Role of Mitogens in Immunobiology. New York, Academic Press, (in press).

15. Hansen, H. H., Selawry, O. S., Carr, D. T., Van Wyk, C. H., Sealy, R. E. and Simon, R.: Combination chemotherapy of advanced lung cancer-- a randomized trial. Cancer (in press).

16. Kenady, D. E., Potvin, C., Simon, R., and Chretien, P. B.: In vitro effect of thymosin on T cell levels in cancer patients receiving radiation therapy. In Proceedings of the Second Conference on Modulation of Host Resistance in the Prevention or Treatment of Induced Neoplasia

17. Kenady, D. E., Simon, R. and Chretien, P. B.: Effect of thymosin in vitro on T cell levels during radiation therapy: Correlations with radiation portal and dose, tumor histology, and initial T cell levels. Am. J. Radiol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE (PROJECT NUMBER (Do NOT use this space))	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-03403-11-M

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Clinical Trials and Miscellaneous Clinical Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Robert C. Young, Chief, Medicine Branch, COP, DCT, NCI
Senior Investigators, MB, COP, DCT, NCI: Richard A. Bender, Bruce A. Chabner, Tom Anderson, Richard I. Fisher, Marc Lippman, Joan Bull, Charles Myers, Douglass Tormey.
Other: Vincent T. DeVita, Director, DCT, NCI
George P. Canellos, Clinical Director, NCI
Stephen H. Rosenoff, Clinical Associate, MB, COP, DCT, NCI
Philip S. Schein, Senior Investigator, MB, COP, DCT, NCI
Stephen Rosenberg, Chief, Surgery Branch, COP, DCT, NCI
Ralph Johnson, Chief, Radiation Branch, COP, DCT, NCI
Costin Berard, Head, Hematopathology Section, LP, NCI
Chemotherapy Research Nurses: Susan Hubbard, Bonny Johnson, Audrey Barlock, Jane Cassidy
Richard Sherins, Senior Investigator, Chemistry Branch, NCI
First Year Clinical Associates, Medicine Branch, COP, DCT, NCI

COOPERATING UNITS (if any)

Radiation Branch, NCI Surgery Branch, NCI Immunology Branch, NCI
Reproduction Research Branch, NICHD

LAB/BRANCH
Medicine Branch

SECTION

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
25	18.5	6.5

SUMMARY OF WORK (200 words or less - underline keywords)

The Medicine Branch is a major clinical facility of the National Cancer Institute. Its activities are divided between clinical therapeutic trials in patients with cancer and clinically related laboratory research. Clinical trials of cancer treatment in the following diseases are currently underway: Breast Cancer, Ovarian Cancer, Hodgkin's Disease, Non-Hodgkin's Lymphomas, Melanoma, Pancreatic Carcinoma, Sarcomas, Chronic Myelogenous Leukemia. In addition Phase I-II clinical trials are underway, studying the following new experimental agents: Gallium Nitrate, Acronicine, 5-Azacytidine, Cytembena, and Maytansine. Laboratory research of the branch is summarized under reports entitled Clinical Pharmacology, Kinetics of Cellular Proliferation, Hematology Studies, Immunological Aspects of Cancer, Hormone Dependence of Malignancy, and Factors Controlling Lymphocyte Reactivity.

PHS-6040
(12-75)

Major Accomplishments in 1975:

Non-Hodgkin's Lymphoma

Published sequential staging techniques which accurately access patterns of spread of the disease. Established the curability of advanced diffuse histiocytic lymphoma with combination chemotherapy. Completed a randomized prospective trial comparing the use of total body irradiation to combination chemotherapy in the lymphocytic lymphomas. Characterized the patterns of relapse after complete remission in the non-Hodgkin's lymphomas.

Ovarian Carcinoma

Established that a high frequency of previously undetected metastatic disease occurs to the diaphragm and can be detected by peritoneoscopy. Published initial results of a randomized prospective trial in advanced ovarian cancer showing a new combination to be more effective than the previous single agent therapy of choice.

Hodgkin's Disease

Published the results of a prospective trial of intensive chemotherapy followed by intensive radiotherapy in advanced Hodgkin's disease and established the risks involved in such therapy. Summarized the existing information on the role of the nitrosourea BCNU in the management of advanced Hodgkin's Disease.

Chronic Myelogenous Leukemia (CML)

Established the activity of Dibromomannitol in the treatment of the chronic phase of CML. Defined the frequency of meningeal leukemia in the blastic phase of CML. Published a randomized prospective trial on the treatment of the blastic phase of CML comparing Vincristine-Prednisone with Cytosine arabinoside-6-thioguanine.

Other major accomplishments in 1975 will be found in the individual project title reports for the Medicine Branch:

Z01-CM-03420-07-M
Z01-CM-06500-03-M
Z01-CM-03404-05-M
Z01-CM-06700-03-M
Z01-CM-06701-03-M
Z01-CM-06702-01-M
Z01-CM-06119-07-M
Z01-CM-03423-05-M

Publications:

1. Young, R.C. and DeVita, V.T.: Ovarian carcinoma: Clinical Trials, prognostic factors and criteria for response. Prognostic Factors and Criteria of Response to Cancer Therapy. Editor, M. Staquet, Brussels, Belgium. P. 319-335, 1975.

2. Rosenoff, S.H., Young, R.C., Anderson, T., Bagley, C., Chabner, B., Schein, P.S., Hubbard, S., and DeVita, V.T.: Peritoneoscopy: A valuable staging tool in ovarian carcinoma. Ann. Int. Med. 83: 37-41, 1975.

3. Young, R.C. The Chemotherapy of Ovarian Carcinoma: Past and Present. Seminars in Oncology 2: 267-276, 1975.

4. Rosenoff, S.H., DeVita, V.T., Jr., Hubbard, S. and Young, R.C.: Peritoneoscopy in the Staging and Follow-up of Ovarian Cancer. Seminars in Oncology 2: 223-228, 1975.

5. DeVita, V.T., Hubbard, S.P., Young, R.C. Trends in the Therapy of Ovarian Cancer. Current Concepts in Diagnosis and Treatment of Intra-Abdominal Cancers. Mount Zion Medical Center Symposium: 203-228, 1975.

6. Young, R.C., Chabner, B.A., Hubbard, S.P., Canellos, G.P. and DeVita, V.T.: Preliminary Results of Trials of Chemotherapy in Advanced Ovarian Carcinoma. National Cancer Inst. Monograph 42: 145-148, 1975.

7. DeVita, V.T., Wasserman, T.H., Young, R.C. and Carter, S.K.: Perspectives on Research in Gynecologic Oncology: Treatment Protocols. Cancer In Press.

8. Rosenoff, S.H., Young, R.C., Chabner, B., Hubbard, S., DeVita, V.T.,Jr., and Schein, P.S.: Use of Peritoneoscopy for Initial Staging and Post Therapy Evaluation of Patients with Ovarian Carcinoma. Nat. Cancer Inst. Monograph 42: 81-86, 1975.

9. Chabner, B.A., Johnson, R.E., Chretien, P., Schein, P.S., Young, R.C., Canellos, G.P., Hubbard, S.H. and DeVita, V.T.: Percutaneous liver biopsy, peritoneoscopy, and laparotomy: An assessment of relative merits in the lymphomas. Brit. J. Cancer 31, Suppl. II, 242-247, 1975.

10. Schein, P.S., Chabner, B.A., Canellos, G.P., Young, R.C., Berard, C., and DeVita, V.T.: Results on combination chemotherapy of non-Hodgkin's lymphoma. Brit. J. Cancer 31, Suppl. II, 465-473, 1975.

11. Canellos, G.P., DeVita, V.T., Young, R.C., Chabner, B.A., Schein, P.S., and Johnson, R.E.: Therapy of advanced lymphocytic lymphoma: A preliminary report of a randomized trial between combination chemotherapy (CVP) and intensive radiotherapy. Brit. J. Cancer 31, Suppl. II, 474-480, 1975.

12. Schein, P.S., Chabner, B.A., Canellos, G.P., Young, R.C. and DeVita, V.T.: Non-Hodgkin's Lymphoma: Patterns of Relapse from Complete Remission after Combination Chemotherapy. Cancer 35: 354-357, 1975.

13. DeVita, V.T., Canellos, G.P., Chabner, B.A., Schein, P.S., Hubbard, S.P. and Young, R.C. Advanced Diffuse Histiocytic Lymphoma, a Potentially Curable Disease. Lancet 1: 248-250, 1975.

14. Ihde, D.C., DeVita, V.T., Canellos, G.P. and Young, R.C. Effect of Splenectomy on Tolerance to Combination Chemotherapy in Patients with Lymphoma. Blood 47; 211-222, 1976.

15. Chabner, B.A., Johnson, R.E., Young, R.C., Canellos, G.P., Hubbard, S.P., Johnson, S.K. and DeVita, V.T. Sequential Non-Surgical and Surgical Staging of Non-Hodgkin's Lymphoma. Ann. Int. Med. In Press.

16. Ihde, D.C. and DeVita, V.T. Osteonecrosis of the Femoral Head in Patients with Lymphoma Treated with Intermittent Chemotherapy Including Corticosteroids. Cancer In Press.

17. Lotz, M.J., Chabner, B.A., DeVita, V.T., Johnson, R.E. and Berard, C.W. Pathological staging of 100 consecutive untreated patients with non-Hodgkin's lymphomas: extramedullary sites of disease. Cancer 37: 266-270, 1976.

18. Young, R.C. and DeVita, V.T. Hodgkin's Disease Current Therapy, 1976. W. B. Saunders Co., Philadelphia, Pa. Edited by H.F. Conn In Press.

19. Kun, L.E., DeVita, V.T., Young, R.C., Johnson, R.E. Treatment of Hodgkin's Disease using Intensive Chemotherapy followed by Irradiation Int. J. of Rad. Onc. In Press.

20. Anderson, T., DeVita, V.T. and Young, R.C. BCNU in the Treatment of Advanced Hodgkin's Disease: Its Role in Remission Induction and Maintenance. Cancer Chemo. Rep. In Press.

21. Young, R.C., Anderson, T., DeVita, V.T. The Treatment of Hodgkin's Disease. Current Problems in Cancer. Year Book Medical Publishers, Chicago, Ill. In Press.

22. Canellos, G.P., Young, R.C., Nieman, P.E., and DeVita, V.T.: Dibromomannitol in the treatment of chronic Granulocytic Leukemia: A Prospective Randomized Comparison with Busulfan. Blood 2: 197-203, 1975.

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24. Canellos, G.P., DeVita, V.T., Whang-Peng, J., Chabner, B.A., Schein, P.S., Young, R.C.: Chemotherapy of the Blastic Phase of CGL Cells - Hypodiploidy and Response to Therapy. Blood. In Press.

25. DeVita, V.T., Young, R.C. and Canellos, G.P.: Combination versus single agent chemotherapy: a review of the basis for selection of drug treatment of cancer. Cancer 35: 98-110, 1975.

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27. Canellos, G.P., DeVita, V.T., Gold, G.L., Chabner, B.A., Young, R.C.: Combination Chemotherapy for Advanced Breast Cancer - Patterns of Response and Effect of Survival. Ann. Int. Med. In Press.

28. Hubbard, S.P., Chabner, B.A., Canellos, G.P., Young, R.C., DeVita, V.T.: High Dose Intravenous Bleomycin in Treatment of Advanced Lymphomas. European J. Cancer 11: 623-627, 1975.

29. Olinger, E.J., McCarthy, D.M., Young, R.C. and Gardner, J.D.: Hyperhistaminema and Hyperchlorhydria in Basophilic Granulocytic Leukemia. In Press.

30. Hubbard, S.P. and DeVita, V.T.: Chemotherapy Research: The Nurse in Oncology. Am. J. Nursing. In Press.

31. DeVita, V.T. and Fisher, R.I.: The Natural History of Malignant Melanoma as Related to Therapy. Cancer Chemo. Rep. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CM-03404-05-M

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (60 characters or less)

Immunologic Aspects Of Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Richard I. Fisher, Senior Investigator, MB, COP, DCT, NCI
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Robert Diasio, Clinical Associate, MB, COP, DCT, NCI
Henry Kaplan, Clinical Associate, MB, COP, DCT, NCI
Daniel Ihde, Senior Investigator, VA, COP, DCT, NCI
William McGuire, Clinical Associate, MB, COP, DCT, NCI

COOPERATING UNITS (if any)

Radiation Branch, NCI V. A. Medical Oncology

LAB/BRANCH
Medicine Branch

SECTION

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to study alterations in the immune system caused by malignancy and chemotherapy. The status of the immune system in Hodgkin's and non-Hodgkin's lymphomas is being evaluated before, during, and after chemotherapy. Immunotherapy of nodular non-Hodgkin's lymphomas with MER will be evaluated and a randomized clinical trial with in vitro monitoring of the immune system is beginning. Animal models are being used to study mechanisms of drug immunosuppression. Infection in immunosuppressed host is being evaluated. A retrospective review of 156 cases of Candidiasis detected at post mortem examination is being completed. The mechanism of action of Pentamidine is being studied in human, bacterial, trepanosomal sources. 5-Fluorocytosine has been shown to be converted to FdUMP in Candida Albicans with resultant depression of thymidylate synthetase activity.

PHS-6040
(12-75)

Publications:

1. DeVita, V.T., Goodell, B., Hubbard, S., Geelhoed, G.W. and Young, R.C.: Pneumocystis Pneumonia in Patients with Cancer: Clinical Setting. J.N.C.I. In Press.

2. Young, R.C. and DeVita, V.T.: The Treatment of Pneumocystis Carinii Pneumonia: Current Status of the Regimens of Pentamidine Isethionate and Pyrimethamine - Sulfadiazine. J.N.C.I. In Press.

3. Fisher, R.I., Jaffe, E.S., Braylan, R.C., Andersen, J.C., Tan, H.K.: Immunoblastic Lymphadenopathy: Evolution into a Malignant Lymphoma with Plasmacytoid Features. Am. J. Med. In Press.

4. Kwon-Chung, K.J., Young, R.C., Orlando, M.: Pulmonary Mucormycosis Caused by Cunninghamella Elegans in a Patient with Chronic Myelogenous Leukemia. Amer. J. Clin. Path. 64: 544-548, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-03420-07-M

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Kinetics of Cellular Proliferation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Charles Myers, Senior Investigator, MB, COP, DCT, NCI
William McGuire, Clinical Associate, MB, COP, DCT, NCI

Other: Stephen H. Rosenoff, Clinical Associate, MB, COP, DCT, NCI
Joan Bull, Senior Investigator, MB, COP, DCT, NCI
Harmar Brereton, Senior Investigator, RB, COP, DCT, NCI
Stanley Shackney, Officer of the Director, DCT, NCI
Paul Bunn, Clinical Associate, MB, COP, DCT, NCI
Oliver Alabaster, Visiting Fellow, MB, COP, DCT, NCI
John Smyth, Visiting Fellow, MB, COP, DCT, NCI
Paul Cretien, Senior Investigator, SB, COP, DCT, NCI

COOPERATING UNITS (if any)

Laboratory of Clinical Pharmacology, NCI
Radiation Branch, NCI Surgery Branch, NCI

LAB/BRANCH

Medicine Branch

SECTION

Cellular Kinetics Section

INSTITUTE AND LOCATION

Clinical Oncology Program, National Cancer Institute, Bethesda, Md., 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

The primary goal of the Cellular Kinetics Section is to develop a better understanding of the kinetic behavior of normal and tumorous tissues and to use such information to design optimal forms of chemotherapy. Toward this end both animal studies and studies in patients have been undertaken. This year Alterations in DNA synthesis induced by chemotherapy in normal and tumorous tissues have been studied after 5-FU, MeCCNU and 5-azacytidine. Nucleoside pool size alterations have been defined after 5-FU therapy in vivo. Kinetic studies in patients with ovarian cancer after methotrexate therapy and studies in patients with chronic myelogenous leukemia after 5-azacytidine have been carried out. Studies are presently underway studying alterations in DNA synthesis induced by Methotrexate in head and neck tumors. Flow microfluorometry studies of ascites tumor from patients with breast and ovarian tumors have been sequentially analyzed after treatment.

Publications:

1. Rosenoff, S.H., Bull, J.M., Young, R.C.: The effect of chemotherapy on the kinetics and proliferative capacity of normal and tumorous tissues in vivo. Blood 45: 107-118, 1975.

2. Rosenoff, S.H., Olson, H.M. and Young, R.C.: Alteration in DNA synthesis in cardiac tissue and light microscopic and ultrastructural cardiac damage induced by Adriamycin in vivo. Second International Symposium on Adriamycin. Brussels, Belgium. In Press.

3. Rosenoff, S.H., Bostick, F. and Young, R.C. Recovery of normal hematopoietic tissue and tumor following chemotherapeutic injury from cyclophosphamide (CTX): Comparative analysis of biochemical and clinical techniques. Blood 45: 465-475, 1975.

4. Rosenoff, S.H., Brooks, E., Bostick, F. and Young, R.C.: Alterations in DNA synthesis in cardiac muscle induced by adriamycin in vivo: Relationship to fatal toxicity. Biochem Pharm. 24: 1898-1901, 1975.

5. Myers, C.E., Young, R.C., Chabner, B.A., Biochemical determinants of 5-fluorouracil response: The role of deoxyuridylate pool expansion J. of Clin. Invest. 56: 1231-1238, 1975.

6. Rosenoff, S.H., Olson, H.M., Young, D.M., Bostick, F. and Young, R.C.: Adriamycin induced light microscopic and ultrastructural cardiac damage in the mouse: A small animal model of cardiotoxicity. J. Nat. Cancer Inst. 55: 191-194, 1975.

7. Brereton, H.D., Bryant, T. and Young, R.C. Inhibition and recovery of DNA synthesis in host tissues in sensitive and resistant B16 melanoma after Methyl CCNU: A predictor of clinical efficacy. Cancer Res. 35: 2420-2425, 1975.

8. Young, R.C., Goldberg, D., Grotzinger, K.R.: Alterations in ^{3}H-Thymidine incorporation into DNA induced by Methyl CCNU [1-(2-Chloroethyl)-3-(4-Methyl Cyclohexyl)-1-Nitrosourea] in normal and tumorous tissues in vivo. Cell and Tissue Kinetics. In Press.

9. Myers, C.E., Young, R.C. and Chabner, B.A.: Kinetic alterations induced by 5-Fluorouracil in bone marrow, intestinal mucosa and tumor. Cancer Res. In Press.

10. Young, R.C., Kinetic aides to proper chemotherapeutic scheduling: Labeled nucleoside incorporation studies in vivo. Cancer Chemo. Rep. In Press.

11. Young, R.C., Holland, J. and Silver, R.T. The Importance of Cellular Kinetics in Modern Cancer Chemotherapy. Am. J. Med. In Press.

12. Young, R.C., Rosenoff, S.A., Myers, C.E., Brereton, H., and Chabner, B.A. Alterations in DNA Synthesis Induced by Chemotherapeutic Agents In Vivo: Potential Applications to Clinical Treatment Schedules. Growth Kinetics and Biochemical Regulation of Normal and Malignant Cells. In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE (PROJECT NUMBER (Do NOT use this space))

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM-03423-05-M

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

In Vitro Marrow Granulocyte Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:
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H. R. Pinedo, Visiting Fellow, COP, DCT, NCI
D. Zaharko, Head, Pharmacokinetic & Pharmacodynamics Sect. LCP, DCT, NCI
B. Chabner, Senior Invest., MB, COP, DCT, NCI
R. Barr, Visiting Fellow, OD, DCT, NCI
J. Whang-Peng, Head, Cytogenetic Onc. Sect., MB, COP, DCT, NCI
R. Atkinson, Physical Science Project Officer, DCT, NCI

COOPERATING UNITS (if any)
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LAB/BRANCH
Medicine Branch

SECTION

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6	3	3

SUMMARY OF WORK (200 words or less - underline keywords)

The laboratory of in vitro marrow culture is concerned with normal granulopoiesis and granulopoietic regulation, as well as abnormal granulopoiesis in bone marrow disease states such as leukemia, and following chemotherapy in non-marrow neoplastic conditions. During the past year, the in vivo and in vitro effects of methotrexate and the rescue of methotrexate toxicity by citrovorum factor and nucleosides on murine myelopoiesis was examined. A human granulopoietic colony stimulating factor intended for clinical trial in aplastic states was extensively assayed in vitro with human marrow. A limiting problem with the assay is an inability to quantify growth without relying on a human subjective factor deriving from visual counts. A macrotube photoelectric counter has been developed and this machine is being calibrated to values found by visual colony counts on mouse and human marrow. Future projects to study clinical marrow and peripheral blood assays in patients with ovarian carcinoma will continue and studies of the chemotherapeutic effects on granulopoiesis will be extended to include hyperthermia + chemotherapeutic studies.

PHS-6040
(12-75)

Objectives:

1. Importance of Methotrexate Plasma Concentration and Duration of Exposure in Determining Marrow Toxicity during Continuous Infusion.

Mice were continuously infused with methotrexate to achieve plasma concentrations 10^{-5} M - 10^{-9} M using the Zanarko pump. Plasma methotrexate concentrations were monitored and femoral marrows were assayed for in vitro myeloid colony forming units (CFU-C) in the interval from 0-48 hours. The study demonstrated that the rate of marrow cell depletion is a function of plasma methotrexate concentration as well as a function of exposure duration, and that there was evidence for an adaptive recruitment of myeloid precursor cells following maximum depletion of marrow nucleated cells.

2. Interaction of Methotrexate and Citrovorum Factor in In Vitro Culture of Marrow Colony Forming Cells.

The ability of mouse femoral CFU-C to survive exposure to several concentrations of methotrexate and L-isomer citrovorum factor was examined in the semisolid in vitro system. Methotrexate concentrations above 10^{-8} M inhibited colony growth. At low (10^{-7}) methotrexate concentrations citrovorum factor rescues at less than equimolar levels. As the concentration of methotrexate increases, rescue requires equimolarity until high (10-5) methorexate concentrations where citrovorum factor levels must exceed methotrexate concentrations to achieve rescue.

3. Rescue of Methotrexate Toxicity by Citrovorum Factor Compared to Nucleosides.

In vitro mouse bone marrow cultures were used to examine the ability of thymidine and combinations of thymidine with adenosine, inosine, guanosine, and hypoxanthine to rescue methotrexate effect on marrow proliferation compared to citrovorum factor rescue. It was found that effects of high concentrations (10^{-5} M) of methotrexate were rescued by combinations of thymidine plus a purine, either adenosine, hypoxanthine, or inosine, but not by citrovorum factor.

4. Peripheral Blood Null Cell Culture.

Mononuclear cells from whole heparinized blood were depleted of thymus dependent lymphocytes by rosette formation with neuraminidase treated sheep erythrocytes. The non T cells were further separated by velocity sedimentation in a sucrose gradient and a monocyte concentrate was removed. These monocyte concentrates were then plated in the semisolid culture system using HEKS and formed numbers of large granulocytic and macrophage colonies. These colonies were subsequently successfully transplanted to further culture dishes. These in vitro null cells did not form erythropoietic colonies in response to erythropoietin stimulation.

5. Photometric Macrotube Scanning.

A photometric macrotube scanner to assay marrow colony formation has been developed during the past year. This machine is able to detect colony formation by means of a reticulated photometric light scattering effect. The correlative work between macroscopic visual colony counts and the photometric analysis is in process for human marrow, murine marrow, as well as cultured lymphoma and breast cancer cells.

6. Abbott Laboratories (CSA).

Abbott Laboratories have a NCI funded contract to develop and prepare a quantity of human colony stimulating factor for human use in certain types of leukemia and aplastic anemia. This is currently in preparation for filing an IND application to the FDA. Our laboratory in conjunction with the Toronto Cancer Institute has been assaying the preparations for pre-clinical testing.

7. Ovarian Carcinoma Patients.

Patients with ovarian carcinoma exhibit decreased marrow reserve after initial chemotherapy. This marrow failure is poorly understood. Marrows and peripheral bloods were obtained on 11 patients prior to therapy. There was no abnormality in in vitro colony production of the marrow precursor cells initially. The study will be continued with repeat cultures following 6 months of therapy.

Future Course of the Projects:

The Clinical ovarian carcinoma project will continue with follow up marrow cultures. There will be increased numbers of assays for the Abbott human CSA.

Hyperthermic toxicity studies examining the effect of heat alone and heat + adriamycin, methotrexate, and cytoxan on mouse and human marrow cells, as well as a human lymphoma cell line (CEM cells), and on human mammary cancer lines will continue. It is expected that the macrotube scanner may be utilized for these experiments in the next few months.

Publications:

1. Bull, J.M., DeVita, V.T. and Carbone, P.P.: In vitro granulocyte production in patients with Hodgkin's disease and lymphocytic histiocytic, and mixed lymphomas. Blood 45: 833-842, 1975.

2. Mabry, J.R., Carbone, P.P., and Bull, J.M.: Amplification of colony stimulating activity in human serum by interaction with CSA from other sources. Exper. Hematol. 3: 354-361, 1975.

3. Bull, J.M., Mabry, R.J., Carbone, P.P. Duttera, M.J., Rosenblum, A.: Studies in granulocyte precursor cell growth in patients with acute leukemia--spontaneous colony formation in acute leukemia. Advances in the Biosciences 14: 215-224, 1975.

4. Bull, J.M. and Chretien, P.: Hyperthermia in cancer therapy. JAMA (In Press).

5. Bull, J., Whang-Peng, J., Carbone, P.P., Dowling, M., and Clarkson, B.: Cytogenetic studies of marrow and peripheral blood granulocyte colonies in treated chronic myelogenous leukemia. Blood Cells 1: 161-162, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CM-06119-07-M

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cytogenetic and Hematopoietic Stem Cell Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jacqueline Whang-Peng	Head, Cytogenetic Oncology, MB COP DCT NCI
Other:	Wind-Shou Cheng	Visiting Fellow, MB COP DCT NCI
	Ronald Barr	Visiting Fellow, OD DCT NCI
	Brigid Leventhal	Senior Investigator POB COP DCT NCI
	Richard Adamson	Branch Chief, Lab. Chem. Pharm. ET DCT NCI
	Susan Sieber	Staff Fellow, Lab. Chem. Pharm. ET DCT NCI
	Harmar Brereton	Senior Investigator ROB DCBD NCI
	Harvey Gralnick	Chief, Hematology, Clin. Path. Dept. CC
	Marvin Lutzner	Chief, Dermatology Branch, DCBD NCI
	Alan Andrews	Senior Investigator, Derm. Branch, DCBD NCI
	Paul Cheng	Baltimore Cancer Research Center
	Ira Green	Senior Investigator, Lab. Immunology NIAID
	Arnold Ratner	Private Practice

COOPERATING UNITS (if any)
Pediatric Onc. Br., COP, DCT, NCI Laboratory of Immunology, NCI
Laboratory of Chemical Pharmacology, ET, DCT, NCI Clinical Pathology Br., NCI
Radiation Onc. Br., NCI Dermatology Br., NCI Epidemiology Br., NCI

LAB/BRANCH
Medicine Branch

SECTION
Cytogenetic Oncology

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

SUMMARY OF WORK (200 words or less - underline keywords)

The areas of investigation are 1) cytogenetic studies of human neoplastic hematological and congenital diseases, and in members of high risk cancer families; 2) the observation of chromosome aberrations and teratogenic effects of drugs such as maytansine, azaribine and Phase I drugs using in vitro and in vivo techniques; 3) the relationship of stem cells, T cells, B cells and null cells in their growth patterns and differentiation; 4) the determination of the existence of stem cells in the peripheral blood of patients with pancytopenia; 5) the detection of chromatid exchanges in patients with xeroderma pigmentosa and in cancer families by use of BrdU and Hoechst stain #33258.

PHS-6040
(12-75)

Major Findings:

1. Cytogenetic studies were performed in 331 cases of ALL, between January 1961 and January 1976. Aneuploidy was observed in the pretreatment marrow in 49/115 (42.6%) of the cases. Aneuploidy in this series exhibited several general characteristics. The aneuploid cells usually co-exist with normal stem cells, hyperdiploidy is predominant, and wide-ranging aneuploidy clusters around a major cell line. The most common chromosomal groups involved are the B and G groups. The appearance of aneuploid cells in the bone marrow at the onset, or later in the disease is of no prognostic significance but persistence of these lines and the development of total aneuploidy signals a poor prognosis.

2. A population of lymphocytes, separable from the great majority, by virtue of their larger size and their failure to exhibit the rosetting characteristics of T and B cells, possess true pluripotentiality. On culture in vitro, these cells proliferate and differentiate into erythrocytic, granulocytic, and megakaryocytic progeny.

3. Chromosome studies were carried out on 11 patients who had the diagnosis of Sezary syndrome. Heteroploidy, and a lack of modality and clone formation were the common chromosomal findings in this syndrome. A final clone formation was the sign of a fulminant process and leads to the terminal phase of the disease.

4. In a study of Guinea Pig L_2C leukemias, it was found that the gene involved in C_3 receptor sites is probably located on the terminal portion of the number 2 chromosome.

Publications:

1. Canellos, G.P., Arseneau, J.C., DeVita, V.T., Whang-Peng, J., and Johnson, R.E.: Second Malignancies Complicating Hodgkin's Disease in Remission. Lancet I: 947-949, 1975.

2. Barr, R.D., Whang-Peng, J., and Perry, S.: Hemopoietic Stem Cells in Human Peripheral Blood. Science 190: 284-285, 1975.

3. Wurster-Hill, D., Whang-Peng, J., McIntyre, O.R., Hsu, L.Y.F., Hirschhorn, K., Modan, B., Pisciotta, A.V., Pierre, R., Balcerzak, S.P., Weinfeld, A. and Murphy, S.: Cytogenetic Studies in Polycythemia Vera. Seminars in Hematology 13: 13-32, 1976.

4. Canellos, G.P., Whang-Peng, J., and DeVita, V.T.: Chronic Granulocytic Leukemia without the Philadelphia Chromosome. Amer. J. of Clin. Path. In Press.

5. Canellos, G.P., DeVita, V.T., Whang-Peng, J., Chabner, B.A., Schein, P., and Young, R.C.: Chemotherapy of the blastic phase of chronic granulocytic leukemia: Hypodiploidy in response to therapy. Blood In Press.

6. Whang-Peng, J., Knutsen, T., Lee, E.C., Leventhal, B.: Acquired XO/XY Clones in Bone Marrow of a Patient with Paroxysmal Nocturnal Hemoglobinuria (PNH). Blood In Press.

7. Whang-Peng, J., Lutzner, M., Edelson, R., and Knutsen, T.: Cytogenetic Studies and Clinical Implications in Patients with Sezary Syndrome. Cancer In Press.

8. Whang-Peng, J., Lee, E.C., Forni, G., and Green, I.: Mutant Lines of Guinea Pig L_2C Leukemia: II. Comparative Cytogenetic Studies and Banding Analyses of Normal and Leukemic Karyotypes. JNCI In Press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CM-06500-03-M

July 1, 1975 through June [illegible] 1976

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of Antineoplastic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Charles Myers, Senior Investigator, MB, COP, DCT, NCI
Richard Bender, Senior Investigator, MB, COP, DCT, NCI
Tom Anderson, Senior Investigator, MB, COP, DCT, NCI
Robert Warren, Clinical Associate, MB, COP, DCT, NCI
Henry Kaplan, Clinical Associate, MB, COP, DCT, NCI
H. M. Pinedo, Visiting Fellow, MB, COP, DCT, NCI

Other: Richard Fisher, Senior Investigator, MB, COP, DCT, NCI
Stephen Come, Clinical Associate, MB, COP, DCT, NCI
Ronald Stoller, Clinical Associate, LCHPH, EXT, NCI
Richard Adamson, Chief, Laboratory of Chemical Pharmacology, EXT, NCI
Robert Dedrick, Acting Chief, Division of Research Services, BEI, NIH
Robert Diasio, Clinical Associate, MB, COP, DCT, NCI
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COOPERATING UNITS (if any)

Laboratory of Chemical Pharmacology, EXT, NCI
Division of Research Services, BEI, NIH

LAB/BRANCH
Medicine Branch

SECTION
Clinical Pharmacology Section

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, Bethesda, Md. 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12	8	4

[illegible] (250 words or less - underline keywords)

A variety of projects are underway to improve the safety and efficacy of therapy with cancer chemotherapeutic agents, including antifolates, nitrosoureas, adriamycin, and 5-fluorouracil. Pharmacokinetic studies have allowed the use of high dose methotrexate with little toxicity, with the help of monitoring of plasma levels and adjustment of leucovorin dosage. In vitro studies are being conducted to elucidate the possible role of drugs which expedite methotrexate transport. The biological properties of nitrosourea derivatives are under study and a new non-myelosuppressive analog, chlorozotocin, will enter trials in the coming year. Methods are being developed to assay a variety of drugs by competitive binding techniques, and these methods are being used to examine the physico-chemical features of binding of substrate and inhibitor in detail.

[illegible]

Project Description:

The Section of Clinical Pharmacology of the Medicine Branch is concerned with studying the mechanism of action, disposition, and pharmacologic correlates of toxicity of established agents, as well as the initial trials of new agents.

Areas of specific interest in the past year include the following:

1. Antifolate Pharmacology

In a co-ordinated effort with the Laboratory of Chemical Pharmacology, routine monitoring of high dose infusions of methotrexate has been provided for patients of the Medicine Branch, as well as other clinical services of the Cancer Institute. This monitoring has led to a record of unusually low toxicity and no fatalities in the co-operative osteogenic sarcoma trial currently underway with the Surgery Branch, NCI. The value of the 48 hour methotrexate level in predicting toxicity has been established. The regimen of urine alkalinization and hydration proposed by our section is now widely accepted as standard for high dose MTX therapy.

We have performed detailed studies of the binding of radio-labelled MTX to dihydrofolate reductase. These studies have led to the design of clinically useful assays for MTX, Baker's antifolate, Methasquin, and Amethopterm. (PNAS 72, 3683, 1975 and Cancer Chemother. Rep. in press). In addition, they have allowed detailed thermodynamic analysis of MTX binding to human DHFR which are now nearing completion.

Additional studies in ovarian cancer patients have provided a tentative understanding of the slow equilibration of MTX in ascites with plasma, emphasizing the role of limited diffusion surface, volume of ascites, and drug permeability in determining the speed of drug exchange. These data have suggested new approaches to treatment of ovarian cancer by the intraperitoneal route.

Other studies (see Annual Report of Laboratory of Chemical Pharmacology, Biochemical Pharmacology Section) have defined the interplay of drug concentration and duration of tissue exposure in determining the extent of cytotoxicity of MTX for normal bone marrow cells. Also documented for the first time was recruitment of stem cells to myeloid colony formation in response to MTX cytotoxicity during high dose MTX infusion.

This section has also been interested in the transmembrane movement of methotrexate (MTX) in neoplastic cells and its augmentation by the concomitant administration of vincristine (VCR). Studies are now in progress to examine the *in vitro* threshold extracellular concentration of VCR necessary to achieve this effect in several human tumor cell lines. Data accumulated to date suggests that the threshold for VCR augmentation is much higher in the tumor cell lines in continuous *in vitro* culture than in blast cells from patients with either acute lymphocytic or myelocytic leukemia. The VCR effect is also being studied

in vivo in mice bearing L1210 leukemia. Cellular uptake studies performed concurrently reveal no in vivo enhancement of MTX uptake using VCR at a dose of 0.15 mg/kg given either 120, 60, 30 or 0 minutes prior to MTX administration. Lastly, we are presently completing a collaborative study which examines the correlation between exchangeable intracellular MTX concentrations, DNA synthesis as examined by deoxyuridine incorporation, and dihydrofolate reductase (DHFR) activity in the L1210 system. Deoxyuridine incorporation is maximally inhibited at an intracellular MTX concentration of 2.6μM corresponding to an extracellular level of 3μM.

Clinically, we are beginning a collaborative study in association with the Experimental Therapeutics Program to examine the human pharmacology of vincristine employing radio-labelled drug.

2. Nitrosourea

It is the goal of this laboratory to pursue the investigation of structure-activity characteristics of the various nitrosourea anti-tumor agents. Previous work has demonstrated that molecular manipulation can alter the ability of these compounds to produce permanent diabetes in animal models, depending upon the characteristics of the alkyl side chain. Further work has demonstrated that by altering the opposite end of the molecule one can minimize bone marrow toxicity in animal models. One such compound, Chlorozotocin, was synthesized under the direction of this section and has no significant bone marrow toxicity. It is about to enter phase I human trials. Current work in this section is related to further investigations to try to identify the pharmacological effects of this compound and its analogs on DNA synthesis, protein synthesis, and lymphocyte function.

3. Pentamidine Mechanism of Action

Pentamidine has been shown to be an irreversible inhibitor of thymidylate synthetase from trypanosomes, bacteria and at least one human tumor line. Structure activity relationships indicate the diamidine structure is essential and a 5 carbon chain length optimal.

4. 5FC Mechanism of Action Against Pathogenic Candida Albicans

5FC exposure led to FdUMP formation within Candida albicans. FdUMP was shown to inhibit Candida albicans thymidylate synthetase in a cell free system. Finally, FC exposure led to a drop in thymidylate synthetase concentration in Candida albicans. It is concluded that FC inhibits Candida albicans growth by conversion to FdUMP and inhibition of thymidylate synthetase.

5. Binding of FdUMP to Human Thymidylate Synthetase

A new, rapid method for studying binding of FdUMP to thymidylate synthetase has been developed along the lines of the methotrexate binding techniques used earlier. Using this approach we have been able, for the first

time, to detect a class of FdUMP binding sites in human tumor tissue. Work is in progress to characterize this binding.

6. Synthesis of Radio-labelled Anthracycline Derivatives

In order to facilitate studies of adriamycin and daunomycin metabolism currently in progress, we have studied synthesis of radio-labelled compounds. A technique has been developed for facile bromination of the 14 position followed by catalytic exchange of tritium for bromine yielding drug with specific activity in the range of 10-100 ci/mmole.

Publications:

1. Pinedo, H.M. and Chabner, B.A.: The Role of Drug Concentration, Duration of Exposure, and Endogenous Metabolites in Determining Methotrexate Cytotoxicity. Cancer Chemotherap. Rep. In Press.

2. Stoller, R.G., Jacobs, S.A., Drake, J.C., Lutz, R.J., Chabner, B.A. Pharmacokinetics of High Dose Methotrexate (NSC-740). Cancer Chemother. Rep. 6: 19-24, 1975.

3. Bender, B.A., Bleyer, W.A., Frisby, S.A., Oliverio, V.T.: Alteration of methotrexate uptake by human leukemia cells. Cancer Res. 35: 1305-1308, 1975.

4. Bender, R.A.: Meningeal carcinomatosis: Treatment with intrathecal methotrexate. Oncology 30: 328-333, 1975.

5. Bender, R.A., Dedrick, R.L.: Cytokinetic aspects of clinical drug resistance. Cancer Chemother. Rep. 59: 805-809, 1975.

6. Dedrick, R.L., Zaharko, D.S., Bender, R.A., Bleyer, W.A. and Lutz, R.J.: Pharmacokinetic considerations on resistance to anti-cancer drugs. Cancer Chemother. Rep. 59: 795-804, 1975.

7. Bender, R.A.: Membrane transport of methotrexate in human neoplastic cells. Cancer Chemother. Rep. 6: 73-82, 1975.

8. Bender, R.A.: Anti-folate resistance in leukemia: Treatment with high dose methotrexate and citrovorum factor. Cancer Treat. Rev. 2: 215-224, 1975.

9. Bender, R.A., Makula, D.R.: Relationship between intra-cellular dihydrofolate reductase and tightly bound intra-cellular methotrexate in human neoplastic cells. Biochem. Pharmacol. In Press.

10. Anderson, T., McMenamin, M.G., Schein, P.S.: Diabetogenic Activity of Deoxy-2 [(ethylnitrosoamino) carbonyl] amino]-D-glucopyranose. Biochem. Pharm. 24: 746-747, 1975.

11. Anderson, T., McMenamin, M.G., Schein, P.S.: Chlorozotocin, 2-[3-(2-Chloroethyl)-3-nitrosoureido]-D-glucopyranose, an Antitumor Agent with Modified Bone Marrow Toxicity. Cancer Res. 35: 761-765, 1975.

12. Schein, P.S., Anderson, T., McMenamin, M.G., Bull, J.: Streptozotocin, Chlorozotocin, and Related Nitrosourea Antitumor Agents. Ninth International Congress of Chemotherapy. 1975. In Press.

13. Myers, C.E., Lippman, M., Eliot, H.M., and Chabner, B.A.: A Competi--tive Protein Binding Assay for Methotrexate. P.N.A.S. 72: 3683-3686, 1975.

14. Myers, C.E., Eliot, H.M., Chabner, B.A.: Competitive Dihydrofolate Reductase Binding Assay of Triazinate, Methasquin and Aminopterin. Cancer Chem. Rep. In Press.

15. Myers, C.E., Diasio, R., Eliot, H.M., Chabner, B.A.: Pharmacokinetics of the Floropyrimidines: Implications for their Clinical Use. Cancer Treat. Rev. In Press.

Objective:

Evaluate kinetics of steroid hormone modulation of lymphocyte replication in vitro.

Methods Employed:

Human lymphocytes are stimulated to grow in tissue culture in the presence or absence of the steroids under study.

Significance to Biomedical Research and Program of the Institute:

This project has broad applicability to problems of altered cell growth, especially cancer and the immunologic system. The elucidation of steroids which physiologically alter the replication of lymphocytes has direct potential application to therapy.

Proposed Course:

The above analysis of steroid kinetics is under investigation.

Abstracts:

1. Neifeld, J.P., Lippman, M.E. and Tormey, D.C. Induction of Glucocorticoid Receptor Activity in Human Peripheral Blood Lymphocytes by PHA Stimulation. Proceedings of the 16th Annual Meeting of the American Society of Clinical Investigation, Atlantic City, 1976.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CM-06700-03-M

PERIOD COVERED
July 1, 1975 through June 31, 1976

TITLE OF PROJECT (50 characters or less)

Clinical Program in Breast Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Douglass C. Tormey, Head, MBCS MB COP DCT NCI
Other: Staff -- Senior Investigators, First and Second Year Clinical Associates, Chemotherapy Nurses -- Medicine Branch COP DCT NCI
Surgery Branch COP DCT NCI:
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M. Cohen, Senior Investigator
P. Chretien, Senior Investigator
T. Pomeroy, Head Radiation Therapy Sect. ROB DCT NCI
D. Henson, Pathologist, Lab. of Path. DCBD NCI
G. Johnston, Chief, Dept. of Nuclear Med. CC
R. Heberman, Chief, Lab. of Immunodiagnosis, DCBD NCI
E. Chu, Head, Cytopathology Sect., Lab. of Path. DCT NCI
R. Simon, Head, Biostatistical Info. Sys. Unit, COP DCT NCI
P. Waalkes, Johns Hopkins University
J. Blom, M.D., Walter Reed Gen. Hosp.
E. Perlin, Bethesda Medical Hosp.
P. Band, Univ. of Alberta, Edmonston, Can.
G. Falkson, H.F. Verwoerd Hosp., South Africa

COOPERATING UNITS (if any) NCI: Surgery Br., Radiation Br., Lab. of Chem. Pharmacol., Tumor Cell Biology Lab., Diagnostic Radiology, Nuclear Medicine, Lab. of Pathology, Cancer Therapy Evaluation Br., Cellular & Tumor Immunology Sect., Therapeutics Projects, Sect., Epidemiology Br.; Contractor Hospitals

LAB/BRANCH
Medicine Branch

SECTION
Medical Breast Cancer Section

INSTITUTE AND LOCATION
Clinical Oncology Program, National Cancer Institute, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10.0	6.0	4.0

SUMMARY OF WORK (200 words or less - underline keywords)

The Medical Breast Cancer Section is responsible for the development of a clinical and laboratory program directed at Breast Cancer. Clinical trials of adjuvant chemotherapy and chemoimmunotherapy, and trials in metastatic disease comparing chemotherapeutic, hormonal and chemohormonal therapeutic regimens are underway. Biochemical and hormonal marker studies are undertaken and coordinated by the Medical Breast Cancer Section.

Project Description:

The Medical Breast Cancer Service was established in July 1972 and the clinical program was initiated in January 1973. It was responsible to the Office of the Associate Director, COP, until its shift to the Medicine Branch in August 1974.

I. Clinical Trials

A. Approach to the primarily diagnosed patient. 7XX

Following radical mastectomy, patients with histopathologically positive axillary lymph nodes are entered into an NCI protocol comparing observation to cyclophosphamide, methotrexate, and 5-fluorouracil with/without C. parvum. L-PAM was substituted for observation in September 1974. 47 entries as of February 1976. It is too early for analysis.

B. Hormone Plus Chemotherapy Trials in Patients with a First Recurrence of Disease. 7XX

1. Premenopausal patients with a measurable first recurrence enter a protocol in which they undergo a bilateral oophorectomy followed by observation for up to 12 weeks. If progressive disease occurs in this interval they enter the second recurrence program (See C.) If a response or no change status is present at 12 weeks, they are then randomized to receive either combination chemotherapy or no further therapy. The latter group will receive the chemotherapy regimen when progressive disease develops. 26 entries as of February 1976. Too early for analysis.

2. Postmenopausal patients with a measurable first recurrence enter a protocol in which they initially receive diethylstilbesterol for 12 weeks. If progressive disease occurs in this interval or no change occurs at 12 weeks, they are then randomized to receive either diethylstilbesterol alone or in combination with chemotherapy. The patients relapsing on the hormone alone arm will subsequently receive combination chemotherapy after progressive disease occurs. 22 entries as of February 1976. It is too early for analysis.

C. Second Recurrence Investigations. 6X1

1. A trial comparing cyclophosphamide, adriamycin and 5-fluorouracil to sequential therapy with combination of adriamycin, dibromodulcitol, vincristine, and cyclophosphamide, methotrexate, 5-fluorouracil was activated in October, 1974. 67 entries as of February 1976. Both regimens are well tolerated. Too early for analysis.

2. A Phase I-II study evaluating dibromodulcitol alone as intermittent therapy has accrued 38 patients. Response rate approximately 20%. The Phase II portion of the study continues.

3. A protocol comparing the anti-estrogen Tamoxifen against Tamoxifen plus Fluoxymesterone had 48 entries. Too early for analysis.

4. A Phase II trial of BCNU and methotrexate is too early for analysis after 9 patient entries.

   A Phase I-II trial of Cytembena for breast and ovarian cancer was closed for breast cancer after no responses among 12 patients.

II. Ancillary Studies

A. Chromosome Analysis

In cooperation with Dr. Jacqueline Whang-Peng and the Surgery Branch we are attempting to provide material serially on patients for chromosome analyses. This project is designed to further our understanding of the disease process and to evaluate the role of chemotherapy in the induction of chromosomal abnormalities.

B. Tumor Marker Analyses

In cooperation with Dr. Phillip Waalkes' laboratory we have supplied blood and urine samples from patients for analysis of tumor markers. The results of this program indicate that 45% to 55% of patients pre- and postoperatively, and 93% of patients with metastatic disease, will have elevations of one or more of the following markers: human chorionic gonadotrophin, carcinoembryonic antigen, or N^2, N^2-dimethylguanosine. We are continuing to evaluate new markers such as casein and ferritin, as well as their relationship to tumor burden, site of lesions, pathology, and response to therapy.

C. Radionuclide Scanning Procedures

The diagnostic importance of brain, liver-spleen, bone, and gallium scans in breast carcinoma have been evaluated systematically. At the present time the data suggests that brain and liver-spleen scanning are not very useful parameters and gallium scanning appears to be a good screening modality only for the detection of mediastinal lesions. Bone scanning is superior to roentgenographic procedures. Recommendations for the use of these procedures have been formulated.

D. Evaluation of Steroid Binding Proteins

Dr. Brad Thompson was receiving tissue from the Surgery Branch and the Medical Breast Cancer Section for the analysis of steroid binding receptors. This project was transferred to Dr. Marc Lippman when he joined our staff in July 1974. A description of the programs can be found in his report.

III. Extramural Activities

The activities listed below have a direct bearing on the program of the Medical Breast Cancer Section in terms of either providing patient material or in integrating the breast cancer program among various investigators.

A. Contract Programs 6X1, 7XX, 5XX

The National Naval Medical Center, Bethesda, and Walter Reed Army Hospital, Washington, are under contract to NCI. Douglass C. Tormey is project officer for these contracts. Both institutions take part in our protocol designs and utilization, thus shortening the duration of time required to complete our studies. To a lesser extent they participate in some of our ancillary study programs.

Dr. Geoffrey Falkson of Pretoria, South Africa and Dr. Pierre Band of Edmonton, Alberta both cooperate with the Medical Breast Cancer Section on a non-contractoral basis to participate in several of the chemotherapy studies.

B. Breast Cancer Task Force 1XX, 7XX

The Medical Breast Cancer Section participates in the Treatment Subcommittee and on the Steering Committee of the Breast Cancer Task Force.

C. Cooperative Group Affiliations 5XX, 5X1, 7XX

The Medical Breast Cancer Section is affiliated with Acute Leukemia Group B, in which Douglass C. Tormey is Study Chairman of two breast cancer studies and is Head of the Breast Cancer Section of the Adult Solid Tumor Committee. Douglass C. Tormey is Chairman of the Breast Tumor Subcommittee of the Eastern Cooperative Oncology Group, and is a Principal Investigator of several Metastatic Disease Protocols in that group.

Publications:

1. Waalkes, T.P., Gehrke, C.W., Zumwalt, R.W., Chang, S.Y., Lakings, D.B., Tormey, D.C., Ahmann, D.L., and Moertel, C.G.: The Urinary Excretion of Nucleosides of Ribonucleic Acid by Patients with Advanced Cancer. Cancer 36: 390-398, 1975.

2. Tormey, D.C., Ellison, R.R., and Hossfeld, D.K.: Concurrent Monoclonal IgM and IgA Proteins in Lymphocytic Lymphoma. Cancer: 36(4), 1321-1326, 1975.

3. Tormey, D.C., and Ingle, J.N.: Diagnostic Considerations in Breast Cancer, Chapter I, in Breast Cancer Diagnosis. (eds. Johnston, G. and Jones, E.) pp 1-17, NY, Plenum, 1975.

4. Tormey, D.C., Waalkes, T.P., Ahmann, D., Gehrke, C.W., Zumwatt, R.W., Snyder, J., and Hansen, H.: Biologic Markers in Breast Carcinoma. I. Incidence of Abnormalities of CEA, HCG, Three Polyamines, and Three Minor Nucleosides. Cancer: 35, 1095-1100, 1975.

5. Neifeld, J.P., Meyskens, F., Tormey, D.C., and Javadpout, N.: The Role of Orchiectomy in the Management of Advanced Male Breast Cancer. Cancer, in press, 1975.

6. Tormey, D.C.: Adriamycin in Breast Cancer: An overview of Studies. Cancer Chemo. Reports 6 (Part 3): 319-327, 1975.

7. Richman, S.D., Brodey, P.A., Frankel, R.S., deMoss, E.V., Tormey, D.C., Johnston, G.S.: Breast Scintigraphy with ^{99m}Tc-Pertechnetate and ^{67}Ga-Citrate. J. of Nuclear Med. 16: 293-299, 1975.

8. Tormey, D.C.: Combined Chemotherapy and Surgery in Breast Cancer: A Review. Cancer 36(3): 881-892, 1975.

9. Tormey, D.C.: Evaluation of Tumor Cell Markers as Indicators of Residual Disease and Response to Therapy. Report to the Profession - Breast Cancer, Sept. 30, 1974 (B. Levine, E. Tarwater, E.P. Vollmer, eds.) Pub. by Breast Cancer Program Coordinating Branch, NCI, Bethesda, 1975, pps. 191-199.

10. Hossfeld, D.K., Tormey, D., Ellison, R.R.: Ph' - Positive Megakaryoblastic Leukemia. Cancer 36: 576-581, 1975.

11. Richman, S.D., Ingle, J.N., Levenson, S.M., Neifeld, J.P., Tormey, D.C., Jones, A.E., Johnston, G.S.: The Usefulness of Gallium Scintigraphy in Primary and Metastatic Breast Carcinoma. J. Nucl. Med. 16: 996-1001, 1975.

12. Thompson, E.B., Perlin, E. and Tormey, D.C.: Steroid Binding Proteins in Human Male Breast Carcinoma Tissue. Amer. J. Clin. Path. Accepted for publication, 1975.

13. Neifeld, J.A., Ingle, J.N., Tormey, D.C., Richman, S.D., Michaelis, L.L.: Mediastinoscopy: A Diagnostic Aid in Metastatic Breast Carcinoma. Cancer, Accepted for publication, 1975.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06701-03-M

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Factors Controlling Lymphocyte Reactivity In Vitro

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I.: Douglass C. Tormey, M.D., Ph.D., Head, Medical Breast Cancer Section, MB, COP, DCT, NCI

Others: James Neifeld, M.D., Clinical Associate, Surgery Branch, COP, DCT, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Medicine Branch

SECTION

Medical Breast Cancer Section

INSTITUTE AND LOCATION

Clinical Oncology Program, National Cancer Institute, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations of the kinetics of steroid hormone modulation of human lymphocyte replication in vitro is in process.

PHS-6040
(12-75)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06702-01M

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mechanisms of Hormone Dependence of Human Breast Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Marc Lippman, Sr. Investigator, MB, COP, DCT, NCI
C. Kent Osborne, Clin. Assoc., MB, COP, DCT, NCI
Marie E. Monaco, NIH Postdoc. Fellow, MB, COP, DCT, NCI
James Neifeld, SB, COP, DCT, NCI

Others: Susan Aitken, Georgetown Univ. Med. School
Walter Griffin, Howard Medical School
E. Brad Thompson, Lab. Biochem. NCI
Ronald Kahn, Diabetes Branch, NIAMDD
Richard Knazek, Lab. Pathophysiol., NCI
Lynn Loriaux, Endocrinology Br., NICHD
Linda Green, Howard Medical School
Ronald Barr, OADP, NCI
Geraldine Konior, Clin. Assoc., POB, NCI
Lawrence Pinkus, N.B., NIAMDD

COOPERATING UNITS (if any) National Institute of Arthritis, Metabolism & Digestive Diseases, Surgery Branch, Lab. Biochem., Diabetes Br., Lab. Pathophysiol., Endocrinology Br., Georgetown Univ. Medical School, Howard Medical School, Pediatric Oncology Br.

LAB/BRANCH

Medicine Branch

SECTION

Medical Breast Cancer Section

INSTITUTE AND LOCATION

Clinical Oncology Program, National Cancer Institute, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.4	4.4	4.0

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the mechanisms whereby steroid and polypeptide hormones stimulate growth and specific protein synthesis in human breast cancer both in tissue culture model systems we have established and in clinical settings.

A. We are studying the prevalency and clinical correlates of specific steroid receptors for estrogen, androgen, glucocorticoid and progesterone in human breast cancer, lymphomatous diseases and melanoma.

B. Assays for specific gene products (α-lactalbumin, CEA, casein, fatty acid synthetase) have been developed and we are studying the effects of steroid hormones and insulin on the activities and synthetic rates of those proteins.

C. We are investigating glucocorticoid receptors in various subpopulations of normal and leukemic lymphoid cells including their regulation by mitogenic stimulation by phytohemaglutinin.

PHS-6040
(12-75)

Publications:

1. Lippman, M.E. Hormonal Receptors in Malignant Disease in Hormones and Cancer. Ed.: K.K. Charyulu Symposia Specialists, Miami, 1976. (In Press)

2. Lippman, M.E. and Bolan, G.A. Estrogen Responsive Human Breast Cancer in Continuous Tissue Culture. Nature 256: 592-593, 1975.

3. Pita, J., Lippman, M.E., Thompson, E.B., and Loriaux, D.L. Interaction of Spironolactone and Digitalis with the 50 Dihydrotestosterone Receptor of Rat Ventral Prostate. Endocrinology 97: 1521-1527, 1975.

4. Lippman, M.E. and Huff, K.: A Demonstration of Androgen and Estrogen Receptors in Human Breast Cancer Using a New Protamine Sulfate Assay. Cancer. (In Press).

5. Neifeld, J.P., Ingle, J.N., Tormey, D.C., Lippman, M.E., Richman, S.D. and Michaelis, L.L. Mediastinoscopy: A diagnostic aid in metastatic carcinoma of the breast. Cancer 1975. (In Press).

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1.0

ANNUAL REPORT OF THE NCI-VA MEDICAL ONCOLOGY BRANCH OF THE DIVISION OF CANCER TREATMENT, NATIONAL CANCER INSTITUTE

1.1
The NCI-VA Medical Oncology Branch is a major clinical facility of the National Cancer Institute and of the Medical Service Washington, D. C. Veterans Administration Hospital (WVAH). The activities of the Branch are divided into: 1) clinical therapeudic trials and clinical investigations into the natural history of malignant disease; 2) Laboratory based investigation in human tumor cell biology;and 3) a service function to the WVAH for the diagnosis and treatment of patients with malignant disease

1.2
The unit is a cooperative venture between the MO/IM/DCT/NCI and the VA with a contract signed between the Director DCT and the VA Central Office (VACO) allowing for the transfer of funds from the DCT/NCI to VACO and the provision of ward, office, outpatient clinic, research space and VA positions to the NCI. Both Veteran and non-Veteran patients are entered into clinical protocals approved by the CRC of the NCI and the Research and Education Committee of the WVAH. A letter of agreement is being drawn up to define the nature, purpose and method of NCI-VA interaction of the unit.

1.3
The clinical studies are conducted by the 6 senior staff, 1 pharmacist, 4 first year clinical associates, 1 second year clinical associate, 2 research nurses and one senior staff on 1/8 time from Georgetown (Dr. Hamosh).

1.4
In conjunction with its clinical investigations and service function to the WVAH the Branch provides for clinical training in the subspecialty of medical oncology for Clinical Associates primarily assigned to it as well as to Clinical Associates rotating from the Medicine Branch and Pediatric Oncology Branch DCT, NCI. In addition, it provides for training in medical oncology to the twelve interns from the Medical Service, WVAH, that rotate through the inpatient.

1.5
The clinical resources of the Branch includes 30 inpatient beds and 3 outpatient clinics a week. The volume of patients studied in the Branch and the variety of disease related increased significantly over the past year. The total patient census is approximately 250.

1.6
Dr. Minna was appointed as the new Branch Chief on July 1, 1975. He has appointed as Deputy Chief of the Branch for Clinical Investigations, Dr. Martin H. Cohen, the previous acting chief

and recruited Dr. Adi Gadzar (previous VLL Section Chief, NCI) the Deputy Chief of the Branch for Laboratory investigation to create and head a Human Tumor Cell Biology Lab. Dr. Daniel Ihde was recruited from the Medicine Branch DCT/NCI to the NCI-VA MOB Senior Staff in July, 1975 and Dr. Paul H. Bunn from the Office of the Director DCT, NCI has been recruited to start full time in July, 1976. A series of 10 new clinical protocols have been designed or adopted, and the area of laboratory investigation completely revised from the previous year. Dr. Oleg Selawry left the Branch in February 1975, and Dr. Patrick Creavan and Larry Allen left in August 1975. The latter investigators had been responsible for the major laboratory work of the Branch in Pharmacology. Their research program Z01 CAM 06509-06 MO was terminated in July-August, 1975 and their references not included in prior annual reports are summarized.

1.7
Summary of Clinical Investigations.
The major clinical effort of the Branch is in the treatment and staging of patients with small cell carcinoma (SCC) within a series of clinical trials. Intensive combination chemotherapy with cyclophosphamide, methotrexate, CCNU (CMC) was shown in a randominzed trial to be more effective than standard (moderate) dose treatment with the same drugs. Over 90% of the patients have an objective response with 30% having a complete response. A randomized trial of the high dose patients has shown that the laminar air flow (LAF) environment has no effect on infectious complications or induction rate. Another study employed combination therapy with vincristine, adriamycin and procarbazine (VAP) in patients relapsing on CMC and showed that 60% of patients would have objective response including some patients with complete responses. We also have looked at patients with prior chest radiotherapy and given them intensive combination chemotherapy. Out of the 10 such patients treated only one has had a clinically beneficial response. This results indicates the importance of initial planning and therapy of patients with SCC.

These studies have lead into the current major study which treats all patients with intensive CMC induction and then randomizes them to receive either alternating cycles of VAP-CMC, or alternating cycles of VAC-CMC with pulses of cell cycle specific agents. Using these regimens over 90% of patients have shown an objective response and 60% of patients have shown a complete response after extensive restaging procedures. This is the highest documented complete response rate known to date.

Several patients with extensive disease treated with chemotherapy alone for periods up to 2 years have been removed from all therapy and remain disease free. This observation is of great therapeutic importance indicating potential chemotherapeutic cure of SCC. Our present studies focus on the intensive staging, combination chemotherapy, documentation of response with emphasis on achieving complete responses after adequate restaging.

We then want to demonstrate this can be translated into increased patient survival.

Trials with combination chemotherapy of adenocarcinoma, large cell carcinoma of the lung and epidermoid carcinoma of the lung are ongoing. While most results are discouraging, a combination of bleomycin, methotrexate and streptozoticin have yielded some bona fide objective responses in these tumor types.

We have found adriamycin to have activity against hepatocellular cancer in American patients but much less than in African patients. This is being prepared for publication.

Collaborative combination chemotherapy protocols with Georgetown division of Oncology including: 5 Fluoruacil, Adriamycin Mitomycin-C for Gastric Carcinoma; 5 Fluouracil, mitomycin-C and Streptozoticin for pancreatic cancer; and 5-Flurouracil, methyl-CCNU, and vincristine for colorectal cancer have yielded a few patients with objective response.

New protocals for combination chemotherapy of prostatic carcinoma, multiple myeloma and other plasma cell dyscrasias have been established. In addition, staging procedures for these diseases will be evaluated.

Phase II trials with VP-16 has shown this to be an active agent in SCC and it will be included in future combinations. Immunotherapy trials with thymosin are beginning in SCC patients receiving combination chemotherapy in collaboration with the Surgery Branch NCI.

We employ fiberoptic bronchoscopy (FOB) in the staging and followup of lung cancer patients and this material is being readied for publication. FOB is an invaluable tool for staging, documentation of objective and complete responses, and identifying early relapses. A comparison of FOB and translaryngeal aspiration as revealed the latter to be a more specific method of obtaining a specimen.

Clinicopathologic correlations for lung cancer are continuing. The importance of classifying patients as small cell or non-small cell carcinoma is emphasized and the need for pathologic expertise in this area being documented.

An intensive, novel approach to the early diagnosis of infection, and determination of the etiology of fever in solid tumor patients undergoing chemotherapy is underway. Most patients receiving intensive therapy develop severe bone marrow toxicity and fever, but only a few have had a documented infection and required antibiotics. Counterimmunoelectrophoresis and development of immunologic reagents for early diagnosis of infections is progressing. ACTA scanning of protocal patients indicate this to be a sensitive, valuable mode of evaluating disease.

1.8
Summary of Laboratory Investigations
The major laboratory investigation of the Branch involves genetic analysis of malignancy using the techniques of somatic cell hybridization. Interspecific hybrids are used to facilitate chromosome, isozyme and malignant product identification, and the chromosome segregation necessary for genetic linkage studies.

RNA tumor virus metabolism in hybrid cells is being intensively studied. Cells permissive for virus replication are fused to non-permissive cells and the hybrids tested for their ability to support exogenously added virus. We are testing primate and non-primate viruses of ecotropic, amphitropic and xenotropic nature as well as viruses that replicate on primate but not on rodent cells. We have found that ecotropic and amphitropic viruses replicate on hybrids but use different genes. Xenotropic viruses do not replicate and thus are controlled by a third set of genes. By introducting xenotropic viruses before fusion we have identified repressor substances acting at a preintegration site.

By using radiolabeled purified viral proteins, we can assay hybrids directly for viral recepotor genes and have found these to segregate independently of other genes important for viral metabolism. We are also examining the spontaneous and induced expression of viruses in hybrid cells and the chromosome localization of endo genous non-expressed virogens with molecular hybridization techniques.

The regulation of viral DNA polymerase by human genes has been discovered in our laboratory and we have found the responsible genes to reside on human chromosomes 12,14,18 and 21.

In sum, these genetic studies of RNA tumor viruses using hybrid cells indicate that we can identify a complex set of genes and assign them to individual chromosomes. These studies will form the foundation for identifying human genes important in the metabolism of RNA tumor viruses.

In related studies we are systematically mapping genes related to carcinogen metabolism. The human genes coding for the structure and regulation of aryl hydrocarbon hydroxylase have been assigned to human chromosome 2 and this material is being prepared for publication.

By fusing non-malignant and malignant cells taken directly from patients we are attempting to identify, localize and then isolate the products responsible for malignant behavior. We take advantage of the segregation of chromosomes in hybrid cells and the ability to select for and against malignant properties (*in vitro* and *in vivo* in nude mice). We examine hybrids before and after such selective pressure and ask which human chromosomes are retained and which are eliminated. These studies will form the foundation for identifying human genes important in

the malignant behavior of cells

We are analyzing human genes coding for DNA synthesis and our first experiments involve the expression of terminal transferase in hybrids formed between acute lymphoblastic leukemia cells and rodent cells not expressing this enzyme. We plan to map this gene.

We have developed techniques to generate hybrids between biopsy specimens from patients early in their disease and grow up large quantities of hybrid cells to use for immunization studies. In preparation for phase I trials we are developing a model system in the mouse involving resection of bulk tumor and then immunization with human x mouse hybrids. Other work has shown hybrid cells to be a more efficient immunogen then tumor cells alone.

The expression of differentiated functions in cultured malignant cells is being explored using transformation of cell lines with oncogenic viruses and by cell fusion. We are trying to identify steps at which cells are blocked in differentiation when they become malignant and to develop means of circumventing this block. At present we have worked with two systems, a virus transformed myoblast line and Friend erythroleukemia cells.

We are routinely inoculating nude (athymic) mice with biopsy samples from our patients in order to generate transplantable cell lines for cell kinetic, in vivo drug sensitivity testing, and for established cultured cell lines. The goal is to have a transplantable tumor line for each patient undergoing chemotherapy and to correlate the response of the tumor in the patient and in the nude mouse. In addition, we are injecting histologically negative specimens to test the nude mouse as an assay system for metastatic disease.

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 03024-07 MO

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Clinical Trials and Miscellaneous Clinical Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John D. Minna	Chief	NCI-VA MOB NCI
	Martin H. Cohen	Deputy Chief (Clin)	NCI-VA MOB NCI
	Daniel C. Ihde	Senior Staff	NCI-VA MOB NCI
	Mary J. Matthews	Senior Staff	NCI-VA MOB NCI
	Bryon E. Fossieck	Senior Staff	NCI-VA MOB NCI
	Albert M. Bernath	Clinical Assoc.	NCI-VA MOB NCI
OTHER:			
	Adi F. Gazdar	Deputy Chief (Lab)	NCI-VA MOB NCI
	Steven Collins	Clinical Assoc.	NCI-VA MOB NCI
	Greg McCormack	Clinical Assoc.	NCI-VA MOB NCI
	William Moss	Clinical Assoc.	NCI-VA MOB NCI
	John Nugent	Clinical Assoc.	NCI-VA MOB NCI
	Frank Pelsor	Pharmacist	NCI-VA MOB NCI
	Paul H. Bunn	Clinical Assoc.	OD/DCT NCI

COOPERATING UNITS (if any)

Hematology and Infectious Disease Sections, Medical Service WVAH, Radiation Therapy Section WVAH, Surgery Service, WVAH. Oncology Division Georgetown University Hospital, Office of the Director

LAB/BRANCH

NCI-VA Medical Oncology Branch

SECTION

None

INSTITUTE AND LOCATION

Washington VA Hospital, Washington, D.C. 20422

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
61	11	50

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to investigate new methods of diagnoses and therapy for patients with malignant diseases. The Therapeutic approach involves combination chemotherapy, combined chemotherapy and radiotherapy, chemotherapy and surgery and recently immunotherapy. The major disease types include: small cell, epidermoid carcinoma, adenocarcinoma and large cell carcinoma of the lung, hepatocellular carcinoma, gastric carcinoma, pancreatic carcinoma colorectal carcinoma, prostatic carcinoma, multiple myeloma and other plasma cell dyscrasias. Phase I and II trials of new agents including VP-16 (NSC 141540) intravenous procarbazine and immunotherapy with Thymosin are underway. Clinical diagnostic studies include: use of fiberoptic bronchoscopy and peritoneoscopy in staging of patients with solid tumors, investigation of fever in patients with solid tumors undergoing chemotherapy, the microflora of the respiratory tree, the early diagnosis of infection by counter-immunoelectrophoresis, evaluation of ACTA scanning and the diagnosis and management of cardiac tamponade. Other investigative efforts involve laminar air flow study to determine the role of protective

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 03024-07 MO

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Clinical Trials and Miscellaneous Clinical Investigations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

OTHER:	Stantley L. Shackney	Investigator	OD/DCT NCI
	Paul Hamosh	Prof. Biophysics	Med. Georgetown
	Geraldine Schechter	Chief Hematology	Section WVAH
	Paul Chretien	Senior Staff	Surg. Branch NCI
	Daniel Kennady	Associate	Surg. Branch NCI

COOPERATING UNITS (if any) (Continued)
DCT, NCI, Surgery Branch DCT, NCI, Dept of Medicine and Biophysics Georgetown Univ.

LAB/BRANCH NCI-VA Medical Oncology Branch

SECTION None

INSTITUTE AND LOCATION
Washington VA Hospital, Washington, D.C. 20422

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
61	11	50

SUMMARY OF WORK (200 words or less - underline keywords)

PHS-6040
(12-75)

2.11 SMALL CELL CARCINOMA

Small cell anaplastic carcinoma (oat cell carcinoma) is a common form (25,000 cases/year USA) of lung cancer. Investigation into the cause, early detection, staging, treatment and mechanisms of resistance to treatment of this malignancy represent the major clinical effort of the branch. A series of clinical trials have been initiated. In the first study, the use of intensive combination chemotherapy in remission induction in small cell carcinoma (SCC) was evaluated. Thirty-three patients were randomized to receive either high dose therapy with cyclophosphamide (CTX) (1000 mg/M^2IV Q 3 weekly), methotrexate (MTX) (15mg/M^2 twice a week), and CCNU (100 mg/M^2IV q6 weeks) or standard dose therapy (CTX 500mg/M^2, MTX 10 mg/M^2, CCNU 50 mg/M^2) on the same schedule. Half of the patients on high dosages were randomized to treatment in a laminar air flow room and all high dosage patients received prophylatic nonabsorbable GI antibiotics. After six weeks all patients were maintained on standard dose therapy. Remission induction was evaluated at 6 and 12 weeks.

On high dose therapy 22/24 (92%) responded with 6 patients (25%) having a complete response after extensive restaging procedures. On the standard regimen 45% of the patients responded with no complete responses. Granulocytopenia, lymphopenia, and thrombocytopenia were significant on the high dose regimen but there was only a single bacterial infection. No difference was detected between patients treated in and out of the LAF room. Median survival has not yet been reached. The conclusions of this study were: that there is a significant dose response for SCC; that patients with this disease can tolerate very intensive chemotherapy and severe bone marrow toxicity with few complications; and that the LAF environment produced no detectable benefit.

The second study involved the use of a combination of vincristine, adriamycin and procarbazine (VAP) in patients who had relapsed on the CMC regimen. Out of 15 patients tested, 60% responded, with several having a complete response. The conclusion of this study was that VAP is a highly active regimine in SCC and is active in previously treated and relapsing patients.

The third study involves a combination of the previous two studies. Patients are extensively staged and all treated with intensive induction therapy (CMC with the doses of CTX increased to 1500/M^2 on day 1).

At 6 weeks the patients are restaged, stratified as to response, performance status and disease extent and randomized to receive alternating cycles of VAP and CMC or to receive a cycle of Vincristine, adriamycin and CTX (VAC) followed by intensive courses of a cell cycle agent such as cystosine arabinoside or other agents such as VP16. Of 15 patients entered 60% have shown a complete response with 93% of the patients showing an objective response when restaged at week 12. The patients tolerated the regimen well despite severe bone marrow toxicity. At present there is no significant difference between the two arms for the response rate at week 15. However, 4 patients treated with cytosine arabinoside all showed disease progression. The median survival has not been reached. The conclusion from this study was that intensive combination chemotherapy with alternating cycles of drugs leads to the highest known complete response rate for chemotherapy alone in SCC. For continuing studies the alternating cycle arm will be continued as a control while the experimental arm will consist of alternating cycles and additional intensive pulses of active drug combination with VP16 (see below).

The fourth study involves attempts at intensive combination chemotherapy in patients previously treated with radiotherapy (XRX) or bed ridden (performance status 4). Of 10 patients treated with intensive CMC followed by vincristine adriamycin combinations, only one has done well. The others have died of complications of their disease or therapy. The conclusion from this study is that such patients are poor candidates for intensive drug therapy and strongly indicates that the initial treatment planning for SCC is crucial for optimal patient survival.

The fifth study involves treatment of patients with SCC and superior vena caval obstruction or airway obstruction with combination chemotherapy alone and no XRX. A group of 8 such patients have been treated; all have responded and done well for at least several months. The conclusion from this study is that SCC and life threatening symptoms can be treated with chemotherapy alone.

The last study involves removing patients from all chemotherapy after a 1.5 to 2.0 year period on continuous therapy. One and possibly 2 such patients who initially had extensive disease have remained disease free for many months to 1 year after stopping treatment. The conclusion from this study is of great conceptual importance for it indicates potential chemotherapeutic cure of SCC.

2.12 ADENO AND LARGE CELL CARCINOMA OF THE LUNG

Adeno and large cell carcinoma which has been notoriously unresponsive to chemotherapy, were treated with a regimen reported to be highly active in adenocarcinoma of the bowel. 5-flurouracil, vincristine and methyl-CCNU (FMV) given in 6 week cycles. Of 15 evaluable patients no objective responses have been found although several disease stabilizations have been seen. The conclusion is that this combination is inactive in adeno and large cell carcinoma of the lung.

2.13 EPIDERMOID CARCINOMA OF THE LUNG

This tumor has also been notoriously unresponsive to chemotherapy. Presently a three drug regimen of individually active drugs was tried: bleomycin, methotrexate and streptozoticin, (BMS). Although only a few patients have been entered, several objective responses have been seen. This study is being continued in better performance status patients.

2.14 HEPATOCELLULAR CARCINOMA (HEPATOMA)

Accrual of patients to this Phase II study of adriamycin (60 mg/M^2 IV q 3 weeks) continues and the results of therapy in the first 12 patients have been analyzed. Only one patient has shown an objective partial response. Pretreatment performance status was found to be an important predictor of survival. We conclude that adriamycin has antitumor activity in American patients with hepatocellular carcinoma, but cannot confirm the high response rates reported in African patients.

2.15 GASTRIC CARCINOMA

In collaboration with Georgetown University Hospital division of Oncology (Dr. P. Schein and J. MacDonald) we have been conducting a Phase II trial of combination drugs each of which is active individually in gastric cancer: 5-Flurouracil, adriamycin, and mitomycin (FAM). Some patients have shown objective responses but these appear to be short lived. Despite other reports of the efficacy of of this regimen we doubt that it will be a standard of treatment.

2.16 In collaboration with the Georgetown Oncology division we are conducting a Phase II trial of combination of single active agents: 3, 5-flurouracil, mitomycin-C and streptozoticin (FMS).

2.17 COLORECTAL CARCINOMA

In collaboration with the Georgetown Oncology division we are conducting a Phase II trial of combination of single active agents already reported to be highly active in colorectal cancer: 5-Flurouracil, methyl-CCNU, and vincristine (FMV).

2.18 PROSTATIC CARCINOMA

Patient accrual to a new treatment program for hormone-unresponsive metastatic carcinoma of the prostate began in March, 1976. Patients will be treated with adriamycin and CTX; after disease progression, the role of further secondary hormonal manipulation wil be investigated. The utility of lymphangiography, liver biopsy and levels of plasma carcinoembryonic antigen in following the course of therapy will be studied.

2.19 MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS

Patient accrual to a new treatment program for multiple myeloma is being opened. Patients are initially treated with melphalan and prednisone to reduce their tumor burden and then placed on alternating cycles of vincristine, adriamycin, cytoxan and melphalan, prednisone and BCNU.

2.20 PHASE II TRIALS WITH VP-16. (NSC 141540)

Patients with SCC who have failed prior combination chemotherapy or radiotherapy were given VP-16. Out of 14 such patients an approximate 30% objective response rate was found. The conclusion from this study is that VP-16 is a highly active agent in SCC and is clinically useful for maintaining patients late in their disease.

2.21 TRIALS WITH INTRAVENOUS PROCARBAZINE

Intravenous procarbazine (compared to oral procarbazine) is not bone marrow toxic, but has CNS side effects. A phase I-II study of this agent in SCC was tried in several patients but only subjective responses have been seen.

2.22 IMMUNOTHERAPY TRIALS WITH THYMOSIN

Thymosin is an extract of calf thymus which can restore aspects of cellular immunity to children with immuno-deficiency disease.

Because there are defects in cellular immunity in patients with lung cancer alone or after treatment we have begun a randomized trial of thymosin vs placebo in patients with SCC receiving intensive combination chemotherapy in collaboration with Dr. P. Chretien and R. Kennady of the Surgery Branch NCI. Patients are followed for T and B Cell level as well as for skin tests.

2.23 SELECTED THERAPEUTIC ASPECTS OF HEMATOLOGIC MALIGNANCIES

Dr. Ihde has analyzed aspects of therapy of malignant lymphoma and chronic granulocytic leukemia (CGL) patients treated by the Medicine Branch, NCI. The appearance of osteonecrosis in lymphoma patients receiving intermittent combination therapy was noted. Chronic phase patients with CGL had no apparent survival benefit from splenectomy. Review of combination chemotherapy in splenectomized and control lymphoma patients revealed no evidence that planned amounts of drugs could be given to treated patients.

2.6 CLINICAL DIAGNOSTIC STUDIES

2.61 LAMINAR AIR FLOW STUDY (LAF)

The LAF study described under SCC revealed no difference in response or infection rate in patients randomized to be treated in or outside the rooms with both groups receiving oral nonabsorbable antibiotics. The only infection occurred in the room. Because of these results, and the need for additional space for clinical work, the immediate plan is to dismantle the LAF units.

2.62 FIBEROPTIC BRONCHOSCOPY

Every patient with lung cancer, or a chest mass is undergoing careful staging fiberoptic bronchoscopy with washings for cytology. For objective measurement tumors are photographed before and after treatment. SCC has a typical appearance (red mass with submucosal infiltrate) and can thus be diagnosed grossly. Patients with superior vena cava obstruction have tolerated fiberoptic bronchoscopy under controlled conditions with washings, brushings and biopsies to obtain material for histologic diagnosis. The conclusions of this study are that fiberoptic bronchoscopy is an invaluable tool in the initial staging and follow-up of patients with chest malignancies.

2.63 CLINICOPATHOLOGIC CORRELATIONS

Dr. M. Matthews, the branch pathologist, has reviewed over 1,000 reference slides, bronchial, liver and bone marrow biopsies, bronchial washings and or sputa and lymph node aspirates on patients entered into or considered for entry into protocols on the NCI-VA MOB. In addition, Dr. Matthews serves as the reference pathologist for the Working Party for Therapy of lung Cancer coordinating and correlating biopsy, cytology and autopsy material on approximately 1,000 patients. She also serves as referee for the VA Surgical Adjuvant lung Group and the VA Surgical Adjuvant Gastric Carcinoma Group. A major effort is being made into classifying lung cancer into small cell and non-small cell varities as well as the subclassification of small cell carcinoma. The major finding to date is that over 90% of patients classified as small cell carcinoma have objective responses to combination chemotherapy and that there is a high percentage of material that is erronously classified as non-small cell carcinoma. This observation will be of great importance in the planning of lung cancer treatment.

2.64 FEVER IN SOLID TUMOR PATIENTS DURING CHEMOTHERAPY

Patients with SCC undergoing intensive combination chemotherapy experience prolonged periods (6 weeks) of severe granulocytopenia and leukopenia. The large majority of these patients also have long periods of fever. We have been following these patients with repeated cultures, and physical examinations. If patients do not demonstrate positive cultures, specific signs of organ dysfunction, or septic shock, they are not given empiric antimicrobial therapy. These patients tolerate their fever well and with the return of granulocytes become afebrile. The major conclusions from this study are that febrile, granulocytopenia patient with solid tumors can be spared the risk of high dose, broad spectrum, empiric antimicrobial treatment. We are presently investigating the etiology of fever in these patients.

2.65 BACTERIOLOGY OF THE LOWER RESPIRATORY TREE OF LUNG CANCER PATIENTS

By performing translaryngeal aspirations followed by fiberoptic bronchoscopy (FOB) in patients being staged for lung cancer, samples have been obtained and cultured to determine the lower respiratory tract microflora.

The lower respiratory tract of 60% of lung cancer patients is infected with bacteria. There is little evidence that these bacteria cause clinical infectious disease. We have found TLA to be the best way to obtain specimens while FOB specimens are misleading (large numbers of samples with multiple organisms). Bacteremia does not accompany FOB in clinically uninfected patients, and TLA was well tolerated. These studies suggest functional impairment of antibacterial systems present in the normal respiratory tree, suggest possible therapeudic approaches and support the use of TLA over FOB for collecting bacteriology specimens.

2.66 EARLY RAPID DIAGNOSIS OF INFECTION IN CANCER PATIENTS

We are systematically trying to develop immunologic methods for the rapid, specific, detection of bacterial, fungal and viral antigens and antibodies in infected hosts. We have found counterimmunoelectrophoresis (CIE) to be a rapid and sensitive method for the diagnosis of staphylococcal infection. The teichoic acid antibody titer is important in characterizing the severity of infection and in guiding the duration of therapy. An optimum CIE system for the detection of C. Albicans antigen and antibody has been developed, and is being tested in an animal model. The major conclusions from this work are the great clinical usefullness of CIE in immunologic diagnosis of bacterial infections.

2.67 ACTA SCANNING (AUTOMATED COMPUTERIZED TRANSVERSE AXIAL) OF CANCER PATIENTS

ACTA scanning is being performed by Dr. Hamosh at Georgetown University on patients with SCC and other selected lesions to document the efficacy of this method in the staging and evaluation of treatments of patients with cancer. Preliminary studies indicate it to be a sensitive, valuable instrument.

2.68 DIAGNOSIS AND MANAGEMENT OF CARDIAC TAMPONADE

IN collaboration with the WVAH cardiology and cardio-vascular surgery divisions we are systematically studying patients presenting with cardiac tamponade. The patients are seen and evaluated by the three units and then taken to the cardiac catheterizations lab. Under controlled conditions a catheter is percutaneously introduced into the pericardical space, and in addition, a right heart catheterization study is performed before and after fluid is removed.

The catheter is left in place for 48 hours and the patient started on appropriate radio or chemotherapy and the fluid examined cytologically. In a series of 10 patients this procedure has been successful in relieving the tamponade for months allowing the patients to resume normal activities and leave the hospital. In addition, the catheterization studies demonstrate that severe impairment of cardiac function occurs despite low levels of paradoxical pulse pressure and normotension. These findings will be of great help in the conservative treatment of patients with this complication.

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PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

ZO1 CM 06309-06 MO

PERIOD COVERED
July 1, 1975 - July 31, 1975

TITLE OF PROJECT (80 characters or less)

Laboratory Studies in Cancer

NAME, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I. Patrick J. Creaven, M.D.
Larry M. Allen, M.D.
Robert L. Nelson, M.D.

COOPERATING UNITS (if any)

None

LAB., BRANCH
NCI-VA Medical Oncology Branch

SECTION
Pharmacology

INSTITUTE AND LOCATION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

SUMMARY OF WORK (200 words or less - underline keywords)

Project terminated with Principal Investigators leaving. References appended.

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Allen, L.M., and Creaven, P.J.: A human pharmacokinetic model of ifosfamide. Proc. Symposium on "Pharmacokinetic Modeling of Anti-Cancer Drugs", Ti Li Look (Ed): Cancer Chemother. Rep. 59:811-817, 1975.

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PROJECT NUMBER (DO NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06575-01 MO

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Laboratory Investigation of Tumor Cell Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Adi F. Gazdar	Deputy Chief (Lab)	NCI-VA MOB NCI
	John D. Minna	Chief	NCI-VA MOB NCI
OTHER:			
	Martin H. Cohen	Deputy Chief (Clinical)	NCI-VA MOB NCI
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COOPERATING UNITS (if any)

Laboratory of Biochemical Genetics NHLI
Viral Leukemia and Lymphoma Branch NCI
Research and Education Service WVAH NCI

LAB/BRANCH

NCI-VA Medical Oncology Branch

SECTION

Human Tumor Cell Biology Laboratory

INSTITUTE AND LOCATION

Washington, VA Hospital, Washington, D. C. 20422

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
18	10	8

SUMMARY OF WORK (200 words or less - underline keywords)

This project investigates tumor cell biology in order to develop new methods for the treatment and diagnosis of patients with malignancy. Our major effort is a genetic analysis of malignancy using somatic cell hybrids, and tumor virology. Interspecific hybrid cells are formed by cell fusion and analyzed by isozymes, chromosome banding, and then tested for properties related to malignancy. These include: RNA tumor virus replication, spontaneous and induced expression of covert genomes, viral receptors, host range and biohazard potential, detection of virogenes by molecular hybridization, expression and regulation of cell and virion DNA polymerase, chromosome segregation, growth in nude mice, contact inhibition, expression of differentiated function, carcinogen metabolism including aryl hydrocarbon hydroxylase, and tumor antigens with immunotherapy trials. Correlation of chromosome content with these properties allows identification and subsequent gene assignment. Other areas include growth of humans tumors in nude mice for cell kinetic and therapy trials, and studies of tumor blood flow and perfusion in experimental tumors.

(continued)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do **NOT** use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Laboratory Investigation of Tumor Cell Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

OTHER:	George J. Todaro	Chief	VLL NCI
	Robert Callahan	Staff Associate	VLL NCI
	J. Delarco	Staff Associate	VLL NCI
	Harry Gelboin	Chief	CH NCI
	Frederick Weibel	Staff Investigator	CH NCI

COOPERATING UNITS (if any) Continue

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LAB/BRANCH

NCI-VA Medical Oncology Branch

SECTION

Human Tumor Cell Biology Laboratory

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

PHS-6040
(12-75)

3.1 GENETIC ANALYSIS OF ONCOGENIC VIRUSES USING HYBRID CELLS.

Interspecific hybrid cells segregating chromosomes provide a method for identifying human genes coding for tumor virus functions. We are first studying RNA tumor viruses. Both murine viruses (MULV,MSV) and primate tropic viruses are studied. Interspecific hybrid cells are generated by fusing human, baboon, mouse, or hamster cells together. Using techniques discussed below, we have discovered how to construct hybrids segregating the desired chromosomes and thus we can study the cellular genes of any species. The hybrid cells are then characterized by isozyme and chromosome banding analysis. The following sets of experiments have been carried out.

3.11 Host cell restriction mechanisms. Cells permissive for virus replication are fused to non-permissive cells and the hybrids tested for their ability to support viral replication. Heterologous cells (such as hamster and human) are restrictive for mouse ecotropic virus. They lack surface receptors for these viruses. The hybrids are permissive for replication provided they have not extensively segregated mouse chromosomes. While large quantities of viral p30 antigen are found in the cytoplasm of hybrid cells infected with ecotropic virus, the amount of infectious virus released is usually low. This suggests some form of packaging problem or an intracellular repressor provided by the heterologous parent cell. In contrast, xenotropic strains of MULV do not replicate at all in human x mouse hybrids indicating that mouse cells have an intracellular repressor substance. We have investigated the possible site of action of this repressor by introducing the viral genome into human cells before fusion. Human cells chronically infected with mouse xenotropic virus (AT124) were fused with spleen cells from N and B-type mice. In 11/12 hybrids, xenotropic virus was expressed, suggesting the repressor acts at a site prior to integration of viral genetic material into the host cell DNA.

3.12 Expression of Surface Receptors for Virus. Hamster cells lack receptors for all known MULV strains. We have studied the replication patterns of nine MULV strains on hybrid cells. Of these, seven were ecotropic strains which replicate only in mouse and rat cells, and two were amphitropic strains from feral mice, which replicate in a wide variety of homologous and heterologous cells. Ecotropic and amphitropic viruses replicated discordantly while the ecotropic strains usually replicated concordantly in hybrid cells suggesting that all ecotropic strains use the same surface receptor while amphitropic strains use a different receptor. The mouse genes coding for expression of these two receptors are probably located on different chromosomes. There was no linkage between the receptor genes and the major murine viral restrictive gene (FV-1) controlling ecotropic replication, or the major ecotropic endogenous structural gene (AKV-1). The major envelope glycoprotein

(gp71) of Rauscher leukemia virus (RLV) was purified by affinity chromatography and iodinated. It absorbed specifically to cells with receptors for RLV. Hybrid cells permissive for RLV replication bound varying amounts of gp71, but never as much as parent mouse cells. Thus, replication of RLV in hamster x mouse hybrids is dependent on expression of surface receptors.

3.13 Spontaneous expression of virus in hybrid cells. Mouse strains vary considerably in the type and ease of spontaneous activation of endogenous virogenes. Thus, AKR cells readily express N-tropic virus and NZB cells xenotropic virus. Virus control is extremely tight in NIH Swiss mice, and intermediate in BALB/c mice. We have the spontaneous expression patterns in hamster x mouse hybrids, to be the same as the parent mouse strains indicating that control of expression is genetically inherited in hybrid cells.

3.14 Induced expression of Virus. We are investigating the induced activation of virus using halogenated pyrimidines known to induce virus in parental cells. Preliminary results suggest that induction in hybrid cells is less indicating a human gene regulating RNA tumor virus expression.

3.15 Chromosome localization of Virogenes. Endogenous virogenes have a specific chromosome localization. We have been looking for the exact site of viral integration using hybrid cells segregating chromosomes. Molecular hybridization is used to determine the presence of the virogene and extensive isozyme and chromosome banding analysis are used to localize it. Using these methods we are mapping the baboon, mouse, and cat virogenes in collaboration with Drs. G. Todaro and R. Calhahan (NCI). Cat and mouse cells have two or more viruses, and thus must have multiple virogenes. These studies can determine if these genes are syntenic (located on the same chromosome) or asyntenic (located on different chromosomes). Using comparative mapping of man with baboon and mouse we hope also to localize putative human virogenes.

3.16 Regulation of viral DNA polymerase by human genes. Mouse and rat cells expressing RNA viruses with high levels of virion associated DNA polymerase (reverse transcriptase) were fused to cells from 12 different humans and the resulting hybrid cells assayed for production of virion associated DNA polymerase and p30 antigen production. One-third of the hybrids expressed virions with greatly reduced levels of DNA polymerase. In contrast, virion p30 antigen was expressed at levels comparable to the mouse parent cell. Isozyme and chromosome analysis of the hybrids with high and low levels of virion DNA polymerase, revealed the low level of expression was associated with a high content of human genes, and specifically a set of genes on human chromosomes 12,14,18 and 21. These studies lay the groundwork for identifying human genes regulating oncogenic viruses.

3.17 Alteration of Viral host range. Because of potential biohazard we have been assaying viruses coming out of hybrid cells for alteration in viral host range. To date, we have seen no examples where ecotropic mouse viruses have acquired

the ability to replicate on human cells.

3.18 Genetic analysis of primate virus replication in hybrid cells. We identify as a separate project the genetic analysis of the metabolism and inheritence of primate type C viruses. Numerous human bone marrow specimens from normal and leukemic patients have been fused to murine and primate cells, dog, cat, baboon, and bat cells. These hybrids are assayed for the production of endogenous primate viruses and their ability to support replication of exogenously added viruses. A wide range of species have been chosen including those known to be permissive for viral replication, In addition, the hybrids can be used for biologic detection of human viruses. S+H- mouse cells carry an integrated, non-replicating sarcoma virus genome which in the presence of a leukemia virus will cause a visable morphologic change in the hybrid cell. This allows for the detection of endogenous human viruses and provides an assay system for exogenously added primate viruses. Exogenously added viruses we are testing include: woolly monkey virus (SSV-1), gibbon ape lymphoma virus (GALV), feline virus strain B (FELV-B), cat xenotropic leukemia virus (CCC), and baboon endogenous virus (M7). These viruses do not replicate in mouse or hamster cells but do so in human cells, and because of the human genes present in the hybrid cells, express surface receptors for them. As the hybrid cells segregate human chromosomes, human genes required for primate virus replication can be identified. Different patterns of replication of the primate tropic viruses have been observed indicating that different human genes are involved. These studies will form the foundation for identifying human genes important in the metabolism of RNA tumor viruses.

3.2 CHROMOSOME SEGREGATION AND COMPARATIVE MAPPING IN HYBRID CELLS.

The mechanism by which preferential chromosome segregation occurs in interspecies somatic cell hybrids is unknown. Most prior studies have only used cultured cell lines. We have studied chromosome segregation in hybrid cells formed by fusing cultured cells of one species with freshly liberated, uncultured, normal differentiated cells of another species. In all examples tested, the chromosomes of the normal tissue donor are segregated even though the opposite pattern is seen when cultured cells of the same parental species are fused. (e.g. human fused to mouse). The mechanism of this elimination process is of fundamental importance for cell biology and indicates there are heritable dirrenences between cultured and freshly liberated cells that determine chromosome replication. We have made use of this preferential elimination of normal tissue chromosomes to perform linkage studies on mouse and Chinese hamster cells. Previously there had been no mechanism of generating interspecies somatic cell hybrids loosing these chromosomes. The method of fusion using normal tissue allowed us to construct such hybrids and we have studied the linkage relationships of over

20 different mouse and Chinese hamster genes. In addition, we have compared the linkage map of man and rodent. In tests involving the isozymes 6-phosphogluconate dehydrogenase, enolase, phosphoglucomutase 1, and peptidase-C all on human chromosome 1, we have found that the genes that are assigned to the same human chromosome arm are linked in the mouse also, while genes assigned to different arms of human chromosome 1 are unlinked in the mouse. These observations are of fundamental importance for understanding the evolution of the genetic map.

3.3 MAPPING OF GENES FOR CARCINOGEN METABOLISM.

We are exploring the genetics of carcinogen metabolism. As a first step, genetic analysis of aryl hydrocarbon hydroxylase (AHH) a typical mixed function oxidase responsible for the catbolism of xenobiotics (drugs, pesticides, and carcinogens) was performed using somatic cell hybridization techniques. This system has been implicated in converting polycyclic hydrocarbons to their carcinogenic forms. In addition, it has been implicated with human lung cancer. Mouse RAG cells express neither constitutive nor induced AHH. These were fused to fresh human bone marrow and hybrids isolated. Induced AHH was expressed in 15/31 hybrid clones. Constitutive AHH was detected in 9/15 inducible clones. Isozyme analysis showed that AHH activity was syntenic with isozymes assigned to human chromosome 2 (malate dehydrogenase and isocitrate dehydrogenase). Isozyme markers for 17 other human chromosomes segregated independently from AHH activity. The results suggest that the structural gene(s) and possibly regulatory genes for AHH are located on human chromosome 2. These observations are fundamental to a detailed understanding of the complex oxidase system, AHH, in mammalian tissues.

3.4 IDENTIFICATION OF GENES CODING FOR MALIGNANT PROPERTIES.

In order to identify genes related to malignancy, we have generated hybrids between rodent cells (mouse, rat and Chinese hamster) and malignant human cells taken directly from patients. The hybrids are grown (in vitro and in vivo) under conditions that select for or against malignant characteristics. The chromosome composition is then determined. Specifically we are asking which human chromosomes are required for malignant behavior and which chromosomes are selectively eliminated in malignant but retained in normal appearing hybrid cells. We have determined so far that chromosome segregation in such hybrids is non-random and that we should be able to identify the chromosomes involved. Such studies will be of importance in mapping human genes responsible for malignancy.

3.5 IDENTIFICATION OF GENES CODING FOR DNA SYNTHESIS.

A systematic analysis of human genes coding for DNA synthesis

is being started using hybrid cells. Terminal deoxynucleotide transferase (TT) is a DNA polymerase important in early development, T cell function, a marker for acute lymphoblastic leukemia, chronic myelogenous leukemia in the blastic phase and in some forms of adult acute leukemia. We have fused human ALL cells expressing high levels of TT with rodent cells expressing undetectable levels of TT. The hybrids are being tested for the expression of TT and gene linkage studies will be performed to determine its chromosome localization. These studies will form the foundation for genetic analysis of DNA synthesis in normal and malignant cells.

3.6 PRECLINICAL IMMUNOTHERAPY STUDIES USING HYBRID CELLS.

We are performing preclinical immunization studies using interspecific hybrid cells in preparation for phase I testing in patients. The advantages of such an approach are twofold: first, large quantities of cells can be generated from small biopsy specimens, and second, the tumor antigens are presented to the host on a different membrane (i.e. hybrid cell) background than the host has previously seen. We have developed methods to take small biopsy specimens, malignant cells from peripheral blood or bone marrow, fuse them to continuous rodent cells, and isolate replicating hybrids in large quantities which can be frozen away for each patient for immunization. Thus, the cells can be prepared at the time of initial biopsy, surgery or at the time of first detection of metastatic disease. Work in other laboratories has indicated in mouse x Chinese hamster hybrids, that tumor cell hybrids are better immunogens than the tumor cells alone. We will show this in a clinically relevant situation for human x mouse hybrids. To establish a model system, we inject mouse tumor cells into a syngeneic mouse, let a tumor with micrometastases develop, and then resect the bulk tumor. The mouse is then immunized with the tumor cells alone, human x mouse tumor cell hybrids, normal human and normal mouse cells. If the method proves effective, it would be possible to derive a panel of such hybrid cells containing a full set of mouse chromosomes but only a few human chromosomes and expression of human tumor related antigens. The benefits of such a system are important for understanding human tumor cell immunology and potentially for immunotherapy.

3.7 EXPRESSION OF DIFFERENTIATED FUNCTIONS IN CULTURED MALIGNANT CELLS.

Because malignant cells are often blocked in the process of differentiation, we have been studying the expression of differentiated functions in cells with and without transformation, and after introducing new genetic information (virus, cell fusion). Cells from normal rat muscle tissue have been established by others. One such line, L5, spontaneously fuses to form multinucleated myotubules. Myotubule formation is accompanied by a great increase in creatine

phosphokinase activity, receptors for acetylcholine, and changes in the composition of myosin. Action potentials for sodium transport are expressed in both myoblasts and myotubules. We have found L5 cells to be highly premissive for the replication of murine Type C viruses. They are readily transformed by murine sarcoma virus (MSV). We have isolated 8 clones of MSV transformed L5 cells. These clones release MSV, grow in soft agar, and are malignant. Under conventional growth condition, the transformed myoblasts do not fuse to form myotubules. Despite the block in differentiation, they express action potentials. We are trying to overcome the block with fusing agents such as polyethylene glycol, or agents which induce differentiation such as dimethylsulfoxide, or by a change in their growth medium. This should be a model system for studying malignant cells blocked in differentiation by specific viral genomes. A second model system we are studying is the differentiation of Friend erythroleukemia cells. These are highly malignant mouse cells which can differentiate along erythroid lines. We have been analyzing hybrids formed between these cells and human cells for the expression of differentiated functions and expression of MULV. The study of differentiated functions has been done in collaboration with Drs. Deisseroth, Neinhuis, and Anderson of Boston and Bethesda. We have found the hybrid cells do not express mouse or human hemoglobin, or hemoglobin messenger RNA, but they have copies of mouse globin DNA in their genome. Thus, the human genes have regulated globin synthesis at or before the level of messenger RNA synthesis. We hope to identify human genes regulating globin expression as the hybrid cells segregate human chromosomes. In addition, we are testing the cells for their malignant potential before and after human chromosome segregation.

3.8 GROWTH OF HUMAN TUMORS IN NUDE (ATHYMIC) MICE.

Mice homozygous for the nude allele on linkage group VII (nu/nu) are characterized by thymic aplasia and will accept heterografts of neoplastic tissue. A variety of human tumors have been propagated in nude mice. A colony of nude mice (nude allele on the BALB/c background) has been established at the NCI-VA MOB. Weanling mice are obtained from the NCI and maintained in a restricted access room. Optimal conditions for longevity are currently being determined. The colony will be used: to assist in the clinical diagnosis and staging of malignant disease. Over thirty bone marrow aspirates and bronchial washings from patients with lung and prostatic carcinoma and acute leukemia have been innoculated into nude mice. The observation periods have been short but several mice have developed nodules at the site of inoculation. The goal will be to have a transplantable tumor line from each patient undergoing chemotherapy. A correlation will be made between response of the tumor in the patient and in the nude mouse. The lines can be used for drug sensitivity testing, testing of drug combinations, and combinations

of chemo and radiotherapy. In addition, we are planning to attempt to correlate cell kinetic studies in the patients with the same tumor growing in the mice. Because *in vitro* lines are easier to establish from *in vivo* transplanted tumors than from primary biopsies, the transplanted tumors provide a useful source for establishing new cell lines in culture. We are injecting bone marrow and bronchial washings from histologically negative as well as positive specimens to determine if the mouse can be used as an assay system for detecting a small number of metastatic cells. This work will have great significance for developing new therapy programs on human tumors outsude the patient. In addition, the tumors can provide source material for immunotherapy studies.

3.9 TUMOR BLOOD FLOW AND ANTINEOPLASTIC AGENT DISTRIBUTION IN SOLID TUMORS.

An experimental model using 591 melanoma in CDF_1 mice has been developed to determine blood flow and drug distribution in a solid tumor. For blood flow determination, bacteria are injected intravenously into the tumor-bearing mouse and clearances are done at various times post injection to compare bacterial levels in normal tissue (spleen, lung, liver) vs. the tumor. For the first 9 hours post injection, bacterial levels in normal tissue are considerably higher than in tumors. After 9 hours, the counts reverse and bacteria in the tumor considerably exceeds those in normal tissue suggesting stasis in the tumor with conditions favoring bacterial growth. For diffusion studies, a sensitive bacteria was placed on an agar petri dish, a drug was injected into the mouse and subsequently tumor imprints on the bacterial plate were made. Numerous non-perfused areas of tumor were noted early. Current studies involve attempts to modify tumor blood flow and diffusion as well as determining the effects of chemotherapy.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06810-12 PO

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Study and Treatment of Cancer in Children and Young Adults

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. S. Levine	Chief, Pediatric Oncology Branch	PO C
	B. G. Leventhal	Head, Chemoimmunotherapy Section	PO C
OTHER:	D. G. Poplack	Senior Investigator	PO C
	P. A. Pizzo	Senior Investigator	PO C
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	R. G. Graw, Jr.	Head, Experimental Hematology Section	PO C
	I. T. Magrath	Visiting Scientist	PO C
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	R. E. Johnson	Chief, Radiation Oncology Branch	R C
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	J. Peng	Head, Cytogenetic Oncology Section	CO C
	R. M. Simon	Head, Biostatistical Inform. Systems Unit	CO C

COOPERATING UNITS (if any)
Surgery Branch, Radiation Oncology Branch, Medicine Branch, Metabolism Branch, Laboratory of Chemical Pharmacology, Immunology Branch (NCI); Reproduction Research Branch (NICHD); Department of Pediatrics (University of Washington)

LAB/BRANCH
Pediatric Oncology Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
30	15	15

SUMMARY OF WORK (200 words or less - underline keywords)

Acute leukemia, non-Hodgkin's lymphomas (especially Burkitt's lymphoma), neuroblastoma, rhabdomyosarcoma, osteogenic sarcoma, histiocytosis and aplastic anemia are studied. In leukemia, we hope to devise effective therapeutic regimens which allow maximum tumor cell kill with chemotherapy and immunotherapy, while leaving intact the immune response. In the solid tumors, we hope to develop combined modality approaches to primary (nonmetastatic) disease. In previously treated (refractory) tumors, our objective is to study the role of high-dose ablative therapy, and the role of supportive care (platelet and white cell transfusion, laminar-flow protection, bone marrow rescue) in permitting such ablation. Phase I trials are also conducted in refractory tumors; agents include maytansine and poly I:C/poly-l-lysine. Research on histiocytosis is concerned with disease mechanisms. Aplastic anemia is studied as a model for bone marrow regeneration kinetics and supportive care techniques. Studies are carried out on the biology of selected tumors, including kinetics, immunology, virology, markers, genetics, and biochemistry. Also studied are the short- and long-term effects of malignant disease and its treatment on growth and development. The psychosocial concomitants of cancer in patients, their families and their therapists are being explored. '619

Project Description:

Objectives:

1) To devise effective therapeutic regimens in acute leukemia which allow maximum tumor cell kill with drugs and with immunotherapy, while leaving intact effective elements of the immune response.

2) To develop effective combined modality treatment approaches to previously untreated childhood solid tumors, including rhabdomyosarcoma, neuroblastoma, non-Hodgkin's lymphoma, and osteogenic sarcoma.

3) To study the possible role of high-dose (ablative) chemotherapy in advanced leukemia and solid tumors, and the role of supportive care (platelet and white cell transfusion, laminar-flow protection, bone marrow rescue, hyperalimentation) in permitting such ablative therapy.

4) To study the biology of selected tumors, including kinetics, immunology, virology, prognostic markers, karyotypes, and biochemistry.

5) To assess the pharmacology of selected atents, e.g., methotrexate, maytansine, and poly I:C/poly-l-lysine, in humans.

6) To study the short- and long-term effects of malignant disease and cancer treatment on growth and development.

7) To study the psychosocial concomitants of cancer in young patients, their families, and their therapists.

Major Findings:

A. Therapeutic Regimens for Acute Lymphatic Leukemia (ALL)

1. Protocol 72-1 was designed to determine whether immunotherapy would increase the effectiveness of chemotherapy in childhood ALL. All patients received POMP, (Prednisone, Oncovin, Methotrexate, G-MP) in 5-day high dose courses until remission was achieved. One group of patients received 4 further POMP courses for bone marrow consolidation and then received prophylactic treatment of the central nervous system. In a second group, the CNS prophylactic treatment was given at the time marrow remission was first achieved, and the CNS treatment was followed with two 5-day courses of Ara-C + 6TG consolidation (this produced marrow hypoplasia in a number of patients). Once patients had completed induction and consolidation, they began maintenance. All patients received four months of treatment (Phase I) with daily 6-MP, weekly methotrexate, and bimonthly pulses of vincristine and prednisone. They were randomized after four months to receive one of two methotrexate schedules, or BCG plus allogeneic cells, during a two month period (Phase II). These two phases were then alternated for a total of 34 months of

maintenance. Seventy patients were entered on this study, which was finished in 1975. Of these, 63 (90%) achieved complete remission and 50% are still in remission at 36+ months. Nineteen have completed all therapy and have been off treatment for up to 18 months. There has been one relapse after three months of unmaintained remission. Although this protocol provided remission durations much longer than those found with previous NIH protocols, there is no significant difference between the three maintenance arms. Thus, immunotherapy with BCG and cells was no more effective than chemotherapy in this regimen.

2. Protocol 75-1. The factors which were associated with a poor prognosis on protocol 72-1 included: failure to achieve complete remission with 4 courses of POMP despite improvement (7/7 relapsed); meningeal leukemia at the time of the first LP (4/5 had subsequent marrow relapse); and an initial white count exceeding 50,000/cu. m. (5/10 relapsed). If these poor prognosis patients are removed from the 72-1 analysis, the projected 40 month remission rate for the remaining group of patients is 80%. Thus, one must be cautious about giving more aggressive therapy to "good prognosis" patients, since additional morbidity from treatment may make long-term remission results worse rather than better. On the other hand, the poor prognosis group is sizable (40% of patients) and for these patients, the 72-1 drug regimen was clearly not adequate. Therefore, for "poor prognosis" ALL patients, one arm of a new protocol (75-1) has been instituted which employs intensive chemotherapy with asparaginase and cytoxan after 4 courses of POMP induction and CNS prophylaxis. In addition, patients are given a full course of Ara-C and daunorubicin as late intensification after six months of remission. Poor prognosis patients on 75-1 are also randomized to receive or not receive the methanol extraction residue of BCG (MER) during induction and maintenance. We wish to determine if nonspecific stimulation of the immune system will result in increased resistance to infection in these heavily treated patients. It is also possible that antitumor immunity might itself be stimulated. To date, nine poor prognosis patients have been entered on 75-1. Seven have achieved complete remission and two are not yet evaluable. Four of the remitters have been assigned to receive MER, of which one has relapsed. Three not assigned to MER have had no relapses. The "poor prognosis" arm of 75-1 is also offered to patients with AML or PDL lymphoma. "Good prognosis" ALL patients are enrolled in a 75-1 arm similar to 72-1.

3. Protocol 74-7. We have previously described the discovery of antigens on the surface of the Burkitt lymphoma cell line RAJI which are shared with acute leukemia blast cells. In a previous report, we described our first therapeutic protocol (73-4) using RAJI immunization. Previously treated patients with ALL in relapse were randomized after reinduction to receive maintenance chemotherapy (COAP) or chemotherapy in combination with RAJI cells (between COAP courses). Five of eight immunized patients made complement-dependent antibody to RAJI and to

autologous and allogeneic leukemia cells. The antibody responses were short-lived, and had no effect on remission duration. The immunized patients did not make lymphocyte-dependent antibody against either RAJI or leukemia cells. Our current relapse protocol (74-7) attempts to broaden and prolong the immune response to RAJI. Remission is induced with asparaginase, and vincristine-daunomycin-prednisone. Patients are then given RAJI between maintenance courses of POMP. Some are then assigned to receive BCG with the RAJI or 1-2 weeks before RAJI. Complement-dependent antibody was found in 2/5 patients who received RAJI alone, 1/4 receiving BCG prior to RAJI, and 3/3 receiving BCG simultaneous with RAJI. The RAJI+BCG group had a rapid and consistent increased in vitro response to RAJI cells. In 11/13 patients, responses were increased over baseline in the first two months after immunization in the BCG group, compared with 3/9 in the non-BCG group. We conclude that BCG has an adjuvant effect with cell vaccines in man, and that simultaneous administration of BCG and RAJI may be the desired schedule.

B. Studies on CNS Leukemia

On protocol 72-1, patients were randomized either to receive prophylactic intrathecal cytosine arabinoside or methotrexate along with 2400 R to the cranial vault. Monthly intrathecal injections were continued for two years. 1/32 patients receiving intrathecal methotrexate and 3/29 on intrathecal cytosine arabinoside have developed meningeal leukemia. Thus, it appears that cytosine arabinoside is effective CNS prophylactic maintenance. The combination of methotrexate and radiation has been toxic to the CNS in some patients, and it was hoped that Ara-C would be less toxic. Unfortunately, two patients receiving this CNS treatment have developed blindness. In one, biopsy of the optic nerve demonstrated atrophy.

For patients with established meningeal leukemia, a protocol was devised (73-6) in which all patients have an Ommaya reservoir inserted and are then randomized to receive, via the reservoir, either a conventional methotrexate regimen of 12 mg/m^2/dose twice a week or a low dose "concentration x time" (CxT) schedule of 1 mg every 12 hours for three days. Nineteen patients have been entered on this study from either NIH or the University of Washington. At a median followup time of 300 days, there were no significant differences between the two treatment groups in the rate of meningeal leukemia remission induction, the number of relapses, the duration of remission or the time on study. Overall, 18/19 patients achieved remission; 2 of 9 on CxT have relapsed and 0 of 9 on conventional doses of MTX. We found a striking difference between the two groups in the incidence of neurologic toxicity during remission (1/9 patients in the CxT group and 7/9 in the conventional dosage group [$p < .05$]). These observations suggest that CxT methotrexate via an Ommaya reservoir may be equally effective against meningeal leukemia as conventional doses, with less methotrexate given and a lower risk of neurotoxicity. A slightly higher dose may be required for optimal CxT treatment than has been the case in the present protocol since both relapses were in the CxT group.

An animal model for CNS pharmacokinetics has been developed. Rhesus monkeys have had Ommaya reservoirs inserted and the pharmacokinetics of methotrexate disappearance appear similar in this model and in man. Acute methotrexate toxicity has been produced, and it is anticipated that this model will be useful in an exploration of toxicity mechanisms.

C. Prognostic Assays in ALL

To facilitate the selection of "poor prognosis" patients for tailored regimens, it is important that new markers be developed to distinguish functional subcategories of lymphoblasts. One suggested prognostic factor has been cell size. Bone marrows were examined in 56 children with ALL treated on the same protocol. Lymphoblasts greater than 12 mu in diameter were called macrolymphoblasts (MLB). Of 56 bone marrows sampled at initial diagnosis, 41 contained less than 20% MLB and 15 more than 20%. Median survival, percent achieving complete remission, duration of first remission, and percent developing CNS leukemia were similar in both groups. Thus there was no correlation between percent macrolymphoblasts at initial diagnosis and prognosis. There was a significant increase in MLB percentage in first relapse marrows (18% median) as compared to initial (pretreatment) bone marrows (8%). This finding correlates with previous observations that a higher percent of cells are in DNA synthesis at the time of relapse than at the time of diagnosis.

We are also examining the surface markers, sheep erythrocyte (E) and erythrocyte antibody complement (EAC') rosette formation, as well as immunoglobulin and receptors for the Fc fraction of the immunoglobulin molecule, on the surface of the lymphoblasts in all patients where these are available for study. Lymphoblasts lacking all these markers are called "null" lymphoblasts. We have found that E rosette-forming blast cells consistently fail to stimulate allogeneic donors in mixed leukocyte culture (11/11 patients). This is a characteristic which these cells share with the normal human T cell. There are, in addition some patients with "null" cell leukemia whose cells do not stimulate in MLC (3/11 patients). We are determining whether this will represent a prognostic marker.

Using previously described techniques, we are also studying the number of steroid binding sites per leukemic blast cell. Nine patients with null lymphoblasts have had relatively high numbers of steroid binding sites per cell (median = 8936). Eight of ten patients with E rosette-forming blasts have had 2500 sites per cell or less. The latter is a value similar to that seen in normal peripheral blood cells. Thus, it appears that null lymphoblasts are more likely to have high numbers of glucocorticoid receptors than E rosette-forming lymphoblasts. Further clinical correlations may be drawn in time.

Another possible prognostic factor is the chromosome pattern of leukemic patients. Dr. J. Whang-Peng has completed an analysis of over 300 cases of acute leukemia studied over a 15 year period. She found that 53% of

the patients showed aneuploidy at some point in their disease. The presence or absence of partial aneuploidy in the marrow was not related to survival. In five patients in whom 100% aneuploidy developed, death occurred within two months. It is of great interest that at least 12 patients developed entirely new clones at some point during their disease. This may imply the development of a second leukemia in these patients.

D. Studies on the Long-Term Effects of Leukemia Chemotherapy on Growth and Development

Pubertal development and reproductive function was studied in 35 female patients of varying ages. Twenty-eight had normal pubertal progression after a median of 74 months since diagnosis of leukemia and 49 months of chemotherapy. Seven patients were abnormal. Four exhibited hypothalamic-pituitary dysfunction with suppression of serum gonadotropins; three others had evidence for primary ovarian dysfunction - reversible in two - with inappropriately elevated serum gonodotropins. Normal sexual development correlated best with patients' pubertal status at onset of leukemia; only one of seventeen patients diagnosed prepubertally experienced altered pubertal progression, whereas abnormalities appeared in 6 of 18 patients with disease onset during puberty or after menarche. Three patients have become pregnant after therapy was discontinued, with delivery of normal infants (one patient who became pregnant while on therapy underwent therapeutic abortion). We conclude that most girls with agressively treated childhood leukemia have an excellent prognosis for normal hypothalamic-pituitary ovarian function.

Similar studies in males are currently being analyzed. It seems that patients who have not received alkylating agents during their therapy have a good prognosis for the preservation of normal sexual development and fertility.

E. Patients with Rhabdomyosarcoma Relapsing with Metastatic Disease During Conventional Chemotherapy

Three patients have been given a regimen of escalating doses of cyclophosphamide in conjunction with adriamycin and DTIC, the endpoint of which is marrow ablation. All three patients have completed the four cycles of the protocol; each has been randomized to laminar-air-flow room isolation for the ablative cycle, and all received autologous marrow rescue. All three patients completed the final ablative cycle without infectious complications, although one developed fatal disseminated CMW weeks later. One patient achieved complete remission on this regimen and remains free of disease nine months later, while a second patient had resolution of pulmonary metastases without an effect on the primary.

F. Utility of Ablative Chemotherapy in Metastatic Neuroblastoma

Our protocol attempts to treat children with metastatic neuroblastoma by utilizing the combination of chemotherapeutic agents heretofore proven

most effective against neuroblastoma, vincristine, DTIC and cytoxan. We are attempting to improve the effectiveness of this conventional chemotherapy combination by giving the cytoxan in sequential cycles of successively higher doses. Vigorous hematologic and antimicrobial supportive care appears necessary to cope with the profound bone marrow toxicity encountered with these high doses of cytoxan. The value of experimental adjuvants such as hyperalimentation and protected environments is being examined. Thus far four patients have entered this protocol. All four have tolerated the three escalatory cycles of chemotherapy. However, two patients died from infectious complications following their final course of therapy. A third patient died with disseminated intravascular coagulation. At post-mortem examination, one patient was completely disease free, one had active disease, and the third has not yet been evaluated. A fourth patient currently is completing his first chemotherapy cycle.

G. Burkitt's Lymphoma

In 1974, several protocols were instituted to study the treatment, immunology, and etiology of American Burkitt's lymphoma. In the first trial (74-0), 15 patients were treated with a combination of cyclophosphamide, vincristine, and methotrexate. All patients but two had advanced disease. After three courses of induction, patients with abdominal tumors received whole abdominal radiation. Following attainment of complete response, patients were then randomized to immunotherapy (BCG and RAJI cells) or to nor further treatment. Of 15 patient entries, there were two early deaths. Thirteen patients achieved complete responses, of whom eight relapsed (seven of the eight patients subsequently died). Major determinants of long survival (five patients) included clinical stage on admission, age (improved prognosis in patients under 12), and interestingly, the antibody titer to virus capsid antigen of the EB virus (higher titer = better survival). Immunotherapy improved the general immunocompetence of the patients in remission and resulted in high titers of anti-RAJI cytotoxic antibodies. This manipulation, however, did not benefit the patient's clinical course. A new protocol (75-6) was begun in June 1975. Prednisone was added to the induction regimen and the third induction course has been replaced by a double dose of cyclophosphamide (45 mg/kg). Induction therapy is followed by whole abdominal radiation to those patients who have abdominal tumor. Thus far, 14 patients have entered this study, all of whom achieved complete remission. Three patients have relapsed at two, three, and four months after induction therapy. While it is too early to ascertain remission duration in this new protocol, the clinical results will be as good or better than with protocol 74-0.

Of considerable interest is the management of non-Hodgkin's lymphoma patients who have relapsed. These patients would ordinarily be treated with Phase I or II agents and would be expected to be dead of their disease within two months of relapse. In 1975, we began to treat such patients with a combination of four agents used for bone marrow transplantation. This combination (BCNU, Ara-C, 6-TG, and cyclophosphamide) is followed by

the infusion of a bone marrow autograft (obtained during remission). Thus far, seven patients have been so treated. Five patients with Burkitt's lymphoma all achieved complete responses; two died early in therapy with drug-associated cardiac toxicity. Modifications of the dose and schedule appear to have prevented serious cardiac toxicity in subsequent patients. Of the three remaining patients with Burkitt's lymphoma, two are now surviving in second or third remissions respectively, 7+ and 14+ months. The third Burkitt patient relapsed soon after the autograft and is not expected to survive. Two patients with histiocytic lymphoma both relapsed within the first two months of their autograft. The long survival of two Burkitt patients in this series who would have been expected to die very quickly of resistant disease is encouraging, and invites this approach in induction therapy to prevent relapse.

A new finding in this series of patients was the observation that 2 out of 14 Burkitt patients harbor the EBV genome in their tumor. This is one of the first discoveries of Epstein-Barr virus-associated Burkitt's lymphoma in a nonendemic region. Other studies on patients with Burkitt's lymphoma include cell surface marker characteristics (all are B cell in origin), studies of chromosome abnormalities (several marker chromosomes have been identified but not the classically described 8-14 chromosome translocation noted in African patients), and the presence of steroid receptors (present in some tumors but not in other).

H. Interferon Inducers as Anti-Tumor Agents

Polyriboinosinic:Polyribocytidylic acid (poly I.poly C), an interferon inducer, was administered in multiple doses of 0.3-75 mg/m^2 to 26 patients with a variety of solid tumors, 9 patients with acute leukemia, and 2 patients with chronic myelogenous leukemia in blast crisis. There were 44 separate drug trials, comprised of various schedules and routes of administration. Toxicities encountered in this study included fever (in 66% of the trials), transient elevation of SGOT and SGPT (25%), minimal laboratory evidence of coagulation abnormalities (59%) and hypersensitivity reactions (5%). These toxic manifestations did not relate to dose level nor magnitude of interferon induction. Poly I.poly C, administered intravenously, induced low serum concentrations of interferon in 24/38 trials (63%), but the correlation between drug dose and peak interferon titer was not linear. Poly I.poly C, administered intravenously or intramuscularly, was not effective as an inducer of interferon in the CSF. Similarly, poly I.poly C administered intramuscularly or by inhalation did not produce detectable serum levels of interferon. No patients experienced an objective tumor response to the administration of poly I.poly C and the majority of patients (76%) had progression of their disease while receiving the drug. A new Phase I-II study has been initiated in 1975 which employs poly I.poly C/poly-l-lysine, a nuclease-resistant complex which may be a more effective inducer in man.

I. Utility of Escalating Chemotherapy (with ablation) in Metastatic Osteogenic Sarcoma

This protocol assesses alternating cycles of adriamycin + cytoxan (AAC) and every-other-day (5 doses) cytoxan (C-C-C-C-C). The AAC doses are progressively increased by adding more consecutive daily cytoxan doses (AAC, AACC, AACCC, etc.). Cytoxan is given at a high dose. Patients not previously treated with methotrexate are also given this agent at a high dose. Ablative cycles may be accompanied by laminar-flow isolation and granulocyte transfusions. To date, five patients have been studied. In three, pulmonary lesions have stabilized, but sepsis supervened (fatal in two). In one, clear progression of metastases occurred. The fifth patient is not yet evaluable. Sepsis was only associated with two or three days of cytoxan (AACC, AACCC); the methotrexate and C-C-C-C-C courses were free of complications.

J. Ablative Chemotherapy for Acute Leukemia

Eight patients with multiple relapses of acute leukemia have been given ablative therapy with a combination of thioguanine, endoxan, Ara-C, and methyl CCNU (TEAM). While 4/8 achieved complete remission, all four subsequently relapsed after a short duration of remission (no maintenance treatment was given). Thus there appears to be no significant benefit associated with this ablative therapy in refractory leukemia. A similar, but not identical, chemotherapeutic regimen (BACT) is given to allogeneic marrow transplant recipients as graft preparation; median leukemia remission in the transplant group has been longer than in TEAM recipients (where evaluable), suggesting the possibility of a transplantation effect beyond that of chemotherapy per se. However, differences in the ablative regimens (BACT vs. TEAM) and small numbers of patients preclude a definite conclusion.

K. Psychosocial Concomitants of Cancer

We have previously reported on the families of patients who have life-threatening illness, and on physicians who care for such patients. In particular, we have explored the factors in the doctor-patient relation which promote anxiety in the doctor to the end that he is less effective than he might be. During the past year, our annual seminar for Clinical Associates on the "Doctor-Patient Relation" has continued on an experimental basis, with the seminar leader an oncologist formally trained in group-process, but not psychotherapy per se. This has been a highly successful experiment (formally the seminar was led by a psychiatrist), and may be widely applicable. We have also initiated therapy groups for parents of ill children and for ill adolescents; these groups are conducted by oncologists and social workers who have received appropriate training in the principles of social and preventive psychiatry. A new research effort has been initiated, in collaboration with the Laboratory of Developmental Psychology, NIMH. The objective of this research is to analyze developmental stages in children who have chronic and/or life-threatening disease,

to learn the factors that promote normal or abnormal development, and the manipulations which may be of value in dealing with developmental abnormalities.

L. Educational Programs

A greatly expanded seminar, lecture, and rounding schedule has been introduced for Clinical Associates and post-doctoral fellows. Two weekly Pediatric Oncology Branch seminars emphasize disease entities in hematology-oncology, and research in the basic sciences related to cancer. Specialized oncology training for nurses is offered by members of the Pediatric Oncology Branch medical staff. The Medical Student Elective program has been highly successful, with a large proportion of students applying for eventual Clinical Associate positions.

Significance to Biomedical Research and the Program of the Institute:

Significant progress is being made in delineating the cell of origin of acute leukemia and in identifying factors which may be useful in predicting response to therapy. By dividing the acute leukemias vis-a-vis these prognostic factors, we hope to individualize therapy to the end that remission rate and duration will further improve. The study of steroid receptors is being pursued in the hope that the basic mechanism of leukemic cell kill can be elucidated. We have learned that the acute leukemia cell has extraordinary antigenic complexity; the effects of both specific and nonspecific immunotherapy in this disease have been discouraging. However, as we evaluate the effects of therapy on general and tumor-specific immunity, it is hoped that our understanding of mechanisms involved in leukemia immune surveillance will increase. The central nervous system is a major site for relapse in acute leukemia. Our studies on route and scheduling of central nervous system chemotherapy promise an effective and relatively nontoxic approach to this important problem. One of our major concerns is the short- and long-term effect of leukemia therapy on growth and development. Our studies on pubertal development and reproductive function have been encouraging in that even long-term therapy seems to be less harmful to the prepubertal patient than to the postpubertal patient.

We are beginning to progress in the treatment of advanced osteogenic sarcoma, rhabdomyosarcoma, neuroblastoma, and Burkitt's lymphoma (non-endemic). A small number of patients with these diseases have been treated with high-dose (ablative) chemotherapy and several have had striking long-term remissions. These studies will be expanded, and maximum supportive care will be offered. Ablative chemotherapy for refractory acute leukemia has been disappointing; while remissions do occur, they are brief.

We have made considerable progress in the development of multi-resource approaches to the psychosocial concomitants of cancer in patients, their families, and the therapists. We believe that this approach is exceedingly important, for failure to deal with these concomitants may be reflected in inadequate treatment. It is our hope that the Pediatric Oncology Branch will provide a model for coordinated research and care in the psychosocial

concomitants of cancer, which will be applicable to many other institutions. Educational programs in pediatric oncology have also been given significant attention and analysis in the hope that these programs also will provide a model for other institutions.

Proposed Course:

All of the studies enumerated previously will be continued. Aggressive protocols are being developed for acute leukemia patients who relapse from primary induction protocols, but remain sensitive to extant chemotherapy. Increased emphasis will be given to Phase I trials. We shall continue to explore the pharmacokinetics of methotrexate and the mechanisms of its instillation so as to improve therapeutic index in the treatment of meningeal leukemia. Experiments in ablative chemotherapy of refractory solid tumors will be expanded; in these studies, chemotherapy will be assessed with maximum supportive care where possible. The utility of laminar-flow-room prophylaxis and granulocyte transfusions will be continually assessed. Two interests will be introduced, with the arrival of new Senior Investigators. Pharmacology will be explored at clinical and molecular levels, and experimental hematology studies will now include the use of somatic cell hybrids to explore erythroid differentiation and leukemia expression. Collaboration has been instituted with the Laboratory of Developmental Psychology, NIMH, and an intensive effort will be devoted to the study of stages in normal and abnormal childhood developmental psychology. The influence on these developmental stages of illness, treatment, and family constellation will be explored.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06820-03 PO

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Blood Component Transfusion and Bone Marrow Transplantation in Cancer and Aplastic Anemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. G. Graw, Jr.	Head, Experimental Hematology Section	PO C
OTHER:	M. I. Bull	Staff Associate	PO C
	R. H. Herzig	Clinical Associate	PO C
	J. C. Kauffmann	Visiting Fellow	PO C
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	J. W. Fay	Clinical Associate	PO C
	A. Gratwohl	Visiting Fellow	PO C
	N. C. Gorin	Guest Worker	PO C
	W. Z. Penland	Head, Bioengineering Unit	DCT C
	G. F. Krueger	Medical Officer	LP C
	H. J. Alter	Chief, Immunology Sect., Blood Bank Dept.	BB CC
	T. C. Pomeroy	Head, Radiation Therapy Section	R C
	H. Y. Reynolds	Head, Bacterial Diseases Section	LCI I

COOPERATING UNITS (if any) Oakridge National Laboratories, Oakridge, Tennessee; Medicine Branch, Radiation Oncology Branch, Laboratory of Pathology, NCI; Blood Bank, Clinical Center, NIH; Laboratory of Clinical Investigation, NIAID; Armed Forces Radiobiology Research Institute, Bethesda, Maryland

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
14	9	5

SUMMARY OF WORK (200 words or less - underline keywords)

In this project, blood components are transfused into patients with depressed bone marrow function; emphasis is on the development of improved procurement and transfusion techniques for platelets and granulocytes. The utility of these various supportive care techniques is assessed in conjunction with chemotherapy and other primary cancer treatment. A second aspect of the project concerns allogeneic, autologous, and isogeneic bone marrow grafting in patients with hematologic malignancies and solid tumors. Various preparative and post-transplant treatments (e.g., anti-thymocyte globulin) are tried in graft-versus-host syndrome. Autologous transplantation is pursued in various solid tumors and non-Hodgkin's lymphomas. In preclinical studies, we examine the effectiveness of combined modality treatment (chemotherapy, radiotherapy, immunotherapy, and supportive care) in lymphosarcoma dogs; the dog is also utilized to investigate new supportive care techniques and bone marrow transplantation.

Project Description:

Objectives:

1) To provide effective blood component transfusion therapy for patients with depressed bone marrow function, with emphasis on the development of improved procurement and transfusion techniques for platelets and granulocytes.

2) To assess methods for obtaining and maintaining allogeneic,autologous, and isogeneic bone marrow grafts in patients with hematologic malignancies and solid tumors.

3) To examine the effectiveness of combined modality treatment (chemotherapy, radiotherapy, immunotherapy and supportive care) in large animals with spontaneous neoplasms (canine lymphosarcoma) and to utilize preclinical animal models (dog) to investigate questions pertaining to supportive care and bone marrow transplantation.

Methods Employed:

Transfusion of platelets and leukocytes in the Clinical Center, NIH, has continued to be the major means of evaluation of new transfusion techniques. Thirteen thousand donors who have been HLA-typed form the available donor pools. A contract facility ("NIH Plateletpheresis Center") has been functioning for 23 months in the plateletpheresis of selected donors, thereby enlarging the capability for delivering selected (non-random) blood products to patients requiring platelet or granulocyte transfusions. Efforts in allogeneic, isogeneic and autologous bone marrow transplantation have been continued for terminal leukemia patients, children with selected solid tumors, and patients with aplastic anemia. Preclinical canine model systems have been utilized for testing transfusion, transplantation, and experimental techniques for combined modality treatment of spontaneous neoplasms.

Major Findings:

A. Blood Component Transfusion Support (Clinical Studies)

1. Platelets. During the past year, 59 patients at the Clinical Center or the Veterans Administration Hospital were supplied with HLA-selected 4-unit platelet concentrates. An additional 18 patients from local Washington hospitals and 8 from hospitals outside the immediate Washington area were also given occasional 4-unit platelet collections. Concurrently, random-donor platelets were supplied to non-alloimmunized patients in the Clinical Center. The NIH Plateletpheresis Center utilized 466 new donors during the year. A total of 41 donor reactions occurred (34-vasovagal; 7 loss of consciousness). An additional 2,695 donors were HLA-typed, bringing the total donor file size to 12,800. A computerized patient transfusion record system was completed and became functional during the year. The system permits integration of

all patient histocompatability and transfusion results. The National Donor Pool has become functional with approximately 24,000 donors now listed. The file includes participation by 13 Centers. Platelets are readily shipped between the various participating Centers and new Centers are being added continuously.

2. Granulocytes. 504 normal granulocyte transfusions were collected for transfusion of patients with suspected or proven bacterial infections. A randomized clinical study of the efficacy of normal granulocyte transfusion was concluded. Twenty-seven patients were randomized on 30 occasions to all arms of the prospectively controlled trial. The results of this study suggest that patients who have persistent granulocytopenia (longer than a median of 8 days) benefit from the short-term administration of granulocyte transfusions. In eight randomizations in which the patient's peripheral WBC failed to recover to 1,000/mm^3 during the period of study, and no leukocytes were transfused, there were no survivors. In a parallel group of 12 patients who were randomized to receive granulocyte transfusions (as well as antibiotics), 9 patients survived. In patients who were transfused with granulocytes but whose WBC recovered to 1,000/mm^3 during the period of study, there appeared to be no benefit to leukocyte transfusions. In this arm of the study, 4 of 5 patients randomized not to receive white cells survived, whereas 4 of 4 patients randomized to receive leukocyte transfusions survived.

   Recently designed centrifuge bowls and an interface controller for the continuous flow-blood cell separator were tested in the laboratory and in human trials. The interface controller was found to substitute adequately for technician time in the optimal operation of the continuous flow-blood cell separator. The new centrifuge bowls, which contain two distinct separating chambers (a red blood cell, leukocyte-rich plasma chamber and a second chamber to concentrate leukocytes from the leukocyte-rich plasma), will be available for initial human trials during May, 1976. These bowls were developed and tested by the ABCOR Corporation and the Oak Ridge National Laboratories.

B. Bone Marrow Transplantation

Four patients were transplanted with allogeneic bone marrow for acute leukemia and five for aplastic anemia during the past year. Four of the nine patients are alive and remain in remission from their disease. The range of remission is 6-9 months. A tenth patient (leukemic) received marrow from an identical twin, and remains in remission at ten months. Three of three evaluable patients with aplastic anemia who were treated with a new combination chemotherapy protocol (procarbazine and cyclophosphamide) achieved excellent engraftments without evidence of rejection. These three patients were restored to normal hematopoiesis and although they have had graft-versus-host disease, they are gradually returning to full activity. The other two aplastic patients died in the immediate post-transplant period (pneumonia, arrythmia). Of the four patients with

acute leukemia, one (with AML) remains in clinical remission at nine months post-transplant, but has suffered recurrent infections associated with graft-versus-host disease. At present, the manifestations of graft-versus-host disease are improving.

A study which investigated the use of anti-thymocyte globulin for the prevention of graft-versue-host disease in allogeneic bone marrow transplantation has been concluded. Four of eight patients treated on the prophylactic schedule are long-term survivors of bone marrow transplantation, although a causal association cannot be concluded. The toxicities of intravenous and subcutaneous ATG administration were found to be similar. Of the nine allogeneic marrow recipients, graft-versus-host disease occurred in all but one patient, who subsequently developed recurrent leukemia. Graft-versus-host was lethal in one patient, and may have contributed to death in two others.

Since 1971, 14 patients with acute leukemia have been transplanted with HLA/MLC-matched sibling bone marrow (10), or marrow from an identical twin (4), using BACT (BCNU, cyclophosphamide, cytosine arabinoside, and 6-thioguinine). Two allogeneic recipients died as a result of a cyclophosphamide-induced cardiomyopathy and were unevaluable for remission duration or survival. The protocol was modified and no cardiac deaths have occurred subsequently. One of the eight evaluable leukemic patients relapsed three months post-transplant. Two of the eight evaluable leukemic patients given allogeneic bone marrow remain in remission at nine months and 4 1/2 years post-transplant. Both had significant graft-versus-host disease and were given ATG. Of the four patients given identical-twin bone marrow, all had uncomplicated transplant courses and swift hematologic and immunologic reconstitution. Three of these patients relapsed at three and four months post-transplant, and died following second attempts to eradicate their leukemia using total body irradiation. The fourth patient remains in clinical remission at 10 months post-transplant. Our total experience with bone marrow transplantation for acute leukemia (allogeneic and isogeneic) has resulted in 6 of the 14 (12 evaluable) patients achieving remission status, and 3 of the 12 continuing in remission at 9, 10, and 54 months post-transplant.

A total of 14 patients have received autologous bone marrow transplants for leukemia, Burkitt's lymphoma, histiocytic lymphoma, and rhabdomyosarcoma. None of the four patients with acute leukemia evidence graft reconstitution following autologous marrow infusion. Two patients with Burkitt's lymphoma remain in unmaintained remission following ablation with BACT chemotherapy for 4+ and 13+ months. Both patients with histiocytic lymphoma have had rapid recurrence of disease despite what was thought to be early reconstitution of peripheral cellular elements following bone marrow infusion. To date, there has been no definite evidence to support the hypothesis that autologous marrow will contribute to the reconstitution of human bone marrow function following preservation at liquid nitrogen temperatures. This finding contrasts with data obtained in our lethally irradiated preclinical canine model, which demonstrated

that rapid reconstitution of bone marrow function occurs following infusion of autologous marrow preserved in liquid nitrogen.

C. Preclinical Studies (See Appendix A.)

Significance to Biomedical Research and the Program of the Institute:

This work consists of research on supportive care techniques which permit the administration of primary cancer therapy. These techniques include platelet administration, granulocyte administration, and bone marrow transplantation. Extensive resources for platelet administration have been developed within the Clinical Center. A national platelet donor resource has also been developed which provides immunologically matched platelets for patients in many Centers. Procurement and transfusion techniques have been improved. Controlled clinical trials have been completed which suggest that normal granulocyte transfusion increases survival from infection in patients with profound bone marrow hypoplasia (granulocytopenia not remitting for several weeks). However, granulocytopenic patients with less profound degrees of bone marrow injury, e.g., those receiving pulses of conventionally dosed chemotherapy, usually recover from serious bacterial infection when treated with systemic antibiotics per se. Patients with profound and enduring bone marrow injury have usually received high doses of ablative chemotherapy as a last resort in the treatment of refractory cancer. In this circumstance, granulocyte transfusion is only as effective as the primary therapy of the underlying disease, and further developments must occur in both primary therapy and granulocyte transfusion pari passu.

The results of our bone marrow transplantation studies in humans with leukemia or aplastic anemia have improved in the past year to the end that projected results are comparable to those of other transplantation Centers. However, only 40 percent of leukemia patients have a donor for immunologically matched transplantation, and of these 40 percent, the long-term survival rate following transplantation has been approximately 20 percent at best. Therefore, allogeneic bone marrow transplantation appears applicable to fewer than 10 percent of patients with leukemia, at present.

The situation in aplastic anemia is somewhat more encouraging in that the long-term survival rate following allogeneic transplantation is approximately 50 percent. Aplastic anemia is under active study in the NCI because it serves as a model for the kinetics of bone marrow regeneration and for supportive care, with applicability to the cancer patient. However, successful transplantation programs require massive resources (personnel, time, effort, money). Both in leukemia and aplastic anemia, allogeneic transplantation must be carefully considered in a Branch whose objective is to study and treat childhood cancer as broadly as possible. On the other hand, autologous bone marrow grafting following cryopreservation is a technique which might be applicable to large numbers of cancer patients, both child and adult, were it to prove technically feasible and of value. Therefore, this area should be actively pursued by the NCI.

Proposed Course:

We shall continue to evaluate new procurement and transfusion techniques for platelets and granulocytes. Of particular interest is the evaluation of donor selection using various histocompatibility criteria, and the relative efficacy of leukocytes collected using various techniques.

We shall continue to evaluate granulocyte antigen typing in prospective donors for leukocyte transfusion.

We shall continue to evaluate autologous bone marrow transplantation procedures both at the preclinical and clinical levels. Of particular interest is the combination of autologous bone marrow rescue with ablative chemotherapy of solid tumors, laminar-flow-room protection, and granulocyte transfusion. Allogeneic transplantation will be de-emphasized.

A new area of interest will be introduced. The proposed work involves the somatic cell hybrid technique, and the objective of the work will be to study the control of erythroid differentiation and the control of leukemia expression using various somatic cell hybrids.

Publications:

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Appendix A

Contract Number: NO1-CM-43702

Contractor: Hazleton Laboratories of America, Inc.

Principal Investigator: Charles Bowles

Budget: $408,580

Man Years: 10 (4 = professional, 6 = other)

Objective:

Preclinical investigation of combined modality treatment of canine lymphoma; leukocyte transfusions; and cryopreservation of bone marrow.

Findings:

1. The canine transfusion model was used to examine the utility of granulocyte transfusions and passive administration of pseudomonas antibody to animals with pseudomonas pneumonia induced after total-body irradiation. These studies indicate that granulocyte transfusion had additive effect in the treatment of pseudomonas pneumonia in animals given prophylactic injections of antibody and antibiotics.

2. Other preclinical studies have addressed the functional characteristics of granulocytes collected by filtration leukapheresis (FL). In animals made granulocytopenic with 40 mg/kg of cyclophosphamide, leukocyte migration into chambers was compared for cells collected with the continuous flow-blood cell separator and cells collected by FL. We concluded that cells collected by FL are defective in their ability to migrate; leukocyte migration with FL cells remained defective even at doses four times higher than those available from the cell separator.

3. A total of 44 dogs with lymphosarcoma were studied. 41% of these animals died either shortly after admission or during the period of induction with CLOP - a combination of chemotherapeutic agents. 25% of the remaining animals completed the study, which consisted of total body irradiation followed by autologous bone marrow infusion. Three of these seven dogs remain alive and in unmaintained remission post-transplant for 200+ - 500+ days. Studies were continued which examine the repopulating potential of bone marrow stored at liquid nitrogen temperatures. Dogs were transplanted with marrow at 9 or 12 months post-marrow harvesting. A large number of dogs in each of these groups have been successfully engrafted with doses of bone marrow which are close to the minimum engrafting dose. These experiments indicate that canine bone marrow can survive long-term preservation using liquid nitrogen storage techniques. A prototype freezing apparatus was evaluated. This new equipment which

utilizes technology developed by NASA engineers permits reproducible freezing curves while minimizing temperature distortion during the heat of fusion. Experiments on repopulation following total body irradiation led to the conclusion that bone marrow stored for short periods of time following cryopreservation with the new freezing instrument was satisfactory.

Plan:

Continuation of studies at a diminished level (230,000/yr.) emphasizing tumor studies, and cryopreservation studies.

Publications: None

Appendix B

Contract Number: NO1-CM-63810

Contractor: Microbiologic Associates

Principal Investigator: Robert Trapani, Ph.D.

Budget: $100,000

Man Years: 3.29 (2.22 = professional)

Objective:

To perform crossmatch studies prior to transfusions of platelets and leukocytes, and to store serum on patients in the Pediatric Oncology Branch.

Findings:

This is a service contract with no new findings.

Publications: None

Appendix C

Contract Number: NO1-CM-02092

Contractor: UCLA

Principal Investigator: Paul I. Terasaki, Ph.D.

Budget: $378,512

Man Years: 12.9 (8.1 = professional)

Objective:

This is a service contract which provides HLA-typing for patients, families, and blood donors for NCI patients (Clinical Center and Baltimore Cancer Research Center). The contract also manages the computer processing required for the national platelet donor pool.

Findings:

See narrative in annual report concerning numbers of donors HLA-typed and the status of the national donor pool.

Publications: None

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PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CM-06830-06 PO

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Infectious Complications of Malignancy: Prevention, Diagnosis and Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	P.A. Pizzo	Senior Investigator	PO C
OTHER:	P.G. Pushkas	Clinical Associate	PO C
	J.O. Reynolds	Clinical Associate	PO C
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	J.D. Maclowry	Chief, Microbiology Service	CP CC
	S.M. Levenson	Staff Fellow	NM CC
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	R.M. Simon	Head, Biostatistical Inform. Systems Unit	CO C
	R. Dolin	Senior Investigator	LCI I
	D.G. Poplack	Senior Investigator	PO C

COOPERATING UNITS (if any)
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
13	8	5

SUMMARY OF WORK (200 words or less - underline keywords)

Infection is the major cause of morbidity and mortality in patients with malignant diseases. We are developing and evaluating the utility of various techniques and procedures to reduce the incidence of severe infection in cancer patients and other compromised hosts. The primary prophylactic technique under study is the laminar-air-flow type of isolation-filtration unit. We wish to learn whether this unit reduces the incidence of infection during chemotherapy, if patients undergoing such protection can tolerate more intensive chemotherapy, and if they consequently have an improved anti-tumor response. Other areas of interest include the improvement of methods by which occult infectious agents may be detected and eradicated; an evaluation of the acquired germ-free state and the influence of this state on physiologic and metabolic processes; and an evaluation of newer anti-viral and anti-fungal agents. In prospective trials, we are assessing the current etiology of febrile episodes in cancer patients, the most appropriate workup for fever in such patients, and the efficacy and toxicity of empiric antibiotic regimens offered for varying periods of time. Other studies concern the radioimmunoassay for candida antigen, the limulus assay for endotoxemia, and the pseudomonas aeruginosa vaccine.

Project Description:

Objectives:

1) To develop and evaluate the utility of various techniques and procedures to reduce the incidence of severe infection in patients with neoplastic diseases and in other compromised hosts.

2) To improve methods by which occult infectious agents may be detected and eradicated.

3) To evaluate the acquired germ-free state and to learn the influence of this state on normal physiologic and metabolic processes.

4) To assess the current etiology of febrile episodes in patients with malignancy and the relationship of these episodes to cancer treatment, prior infection, and antigenic encounters; to determine the most appropriate workup for fever in patients with malignant disease by assessing invasive and noninvasive diagnostic techniques.

5) To assess the efficacy and toxicity of empiric antibiotic regimens offered for varying periods of time.

6) To learn whether a protected environment reduces the incidence of infection during chemotherapy (with or without autologous bone marrow rescue), if patients undergoing such protective regimens can tolerate more intensive chemotherapy, and if they consequently have an improved anti-tumor response.

Methods Employed:

Quantitative evaluation of the efficacy of prophylactic techniques in the compromised host is based upon sampling of the patients and their environment for various microorganisms while such patients are offered a comprehensive antimicrobial regime in a laminar-air-flow isolation-filtration unit.

The studies and treatment of febrile patients with cancer employ the broad range of current medical practice. Newer diagnostic studies include gallium scanning for potential localization of infection, application of the recently developed radioimmunoassay for candida antigen, and clinical application of the limulus assay for endotoxemia.

Major Findings:

A. Lack of Clinical Usefulness of the Limulus Test in the Diagnosis of Endotoxemia

Studies of the clinical value of the limulus amebocyte lysate test for the detection of endotoxemia are inconsistent. In an attempt to define the value of this test, a total of 237 plasma samples from 111 patients were tested for endotoxin with seven different lysate preparations. A

total of 48 plasma samples yielded a positive test with one or more of the seven preparations. Two of eight samples positive with all seven preparations were from ambulatory patients. A significant positive correlation of the test with bacteremia, neutrophilia and elevated serum alkaline phosphatase was found. Only three of the 48 positive tests occurred by four hours of incubation, and only 12 were associated with positive blood cultures (eight contained gram-negative bacteria). The test now available has no clinical usefulness in the detection of endotoxemia or gram-negative septicemia.

B. Use of a Pseudomonas Aeruginosa Vaccine in Patients with Acute Leukemia and Cystic Fibrosis

A heptavalent lipopolysaccharide Pseudomonas vaccine was evaluated in 22 patients with acute leukemia and 12 patients with cystic fibrosis during an 18 month interval. Of the 34 patients, 32 had an excellent serum hemagglutinating (HA) antibody response to immunization. In comparison to the patients with cystic fibrosis, the patients with leukemia had a smaller HA antibody response, which lasted a shorter period of time, and also experienced greater toxicity from the vaccine. The mixing of adrenal corticosteroids with vaccine greatly decreased side reactions among the patients with leukemia without significantly inhibiting antibody production. Previous antineoplastic chemotherapy had little influence on antibody response in patients with leukemia, with the exception of methotrexate. Vaccinated patients with leukemia had 1 Pseudomonas infection of 14 bacterial or fungal infections, whereas 2 Pseudomonas infections of 5 bacterial or fungal infections occurred in a control group of 20 patients with acute leukemia. Of the 12 patients with cystic fibrosis, 4 had a Pseudomonas infection after vaccination.

C. The Relationship of Fever, Granulocytopenia and Antimicrobial Therapy to Bacteremia

The relationship of fever, granulocytopenia, and antimicrobial therapy to bacteremia was studied retrospectively in 53 cancer patients. Granulocytopenia was present at the time blood cultures were positive in 27 of 31 consecutive bacteremias. No bacteremia occurred in the absence of fever. Only 2 bacteremias occurred while patients were receiving parenteral antimicrobials. At the initiation of 76 courses of antibiotics in the presence of granulocytopenia and fever the incidence of bacteremia was 33%. After terminating antibiotics 30 times in the presence of granulocytopenia and fever the incidence of bacteremia was 47%. Patients who died with fungal disease did not receive more antibiotics than patients who died without fungal disease. These data suggest a rationale for long-term prophylactic use of antimicrobial therapy in patients with persistent granulocytopenia and fever.

D. Fever and Antibiotic Study

Approximately 60 patients have been entered in this randomized study since its initiation in November, 1975. The study assess empiric antibiosis and

duration of treatment in granulocytopenic patients with fever of unknown origin. Nearly one-third of the patients have a demonstrable infectious source initially. No conclusions can yet be drawn regarding the natural history or treatment of these patients. The past year has shown a decrease in gram-negative infections and a significant increase in the incidence of staph aureas infections in our patient population.

E. Protected Environment Study

The current randomized protocol assessing the utility of this regimen in patients ungergoing ablative therapy for leukemia or certain solid tumors was initiated in December, 1975. Five patients have now been randomized including two with leukemia, two with sarcomas, and one with neuroblastoma. Of the five patients, two have received the protective regimen and three have been treated in a conventional hospital setting (controls).

Significance to Biomedical Research and the Program of the Institute:

Infection is the major cause of morbidity and mortality in patients with malignant diseases. The incidence of infection appears directly related to the degree and duration of granulocytopenia, and the presence or threat of infection frequently limits the amount and frequency with which chemotherapeutic regimens may be given. It is possible that if the incidence of infection could be reduced, more aggressive therapy might be tolerated and that this therapy would enhance remission and survival rates. Certain pediatric tumors, such as Burkitt's lymphoma, metastatic sarcoma, and neuroblastoma, might be responsive to such aggressive therapy. If so, prophylaxis of these patients from infection by a protected environment might provide a therapeutic approach to these currently unresponsive tumors. Moreover, a proof of tumor responsiveness in such a system would provide a rational utilization for laminar-air-flow technology.

A careful rational approach to the evaluation and treatment of possible or established infection in the compromised host is critical if maximum benefit is to be derived from extant chemotherapy. Systematic analysis of therapeutic antimicrobial combinations, duration of treatment, and the potential efficacy of antimicrobial prophylaxis should provide guidelines for maximizing the supportive care of these complex patients.

Proposed Course:

We shall continue our major studies on fever and antibiosis in the cancer patient, and our assessment of the utility of protected environments in the treatment of various pediatric solid tumors. We shall initiate studies which employ zoster immune globulin prophylaxis of children with malignant disease exposed to varicella; the role of zoster immune globulin in the prophylaxis and treatment of zoster in patients with Hodgkin's disease will also be explored. We shall continue to assess the utility of adenine arabinoside as an antiviral agent in the treatment of varicella and zoster. We shall continue our studies on the limulus assay for endotoxemia and our assessment of anti-

bacterial vaccines in the compromised host. New studies of potential antiviral agents (e.g., the psoralens) will be undertaken.

Publications:

1. Pennington, J.E., Reynolds, H.Y., Robinson, R., Wood, R.E. and Levine, A.S.: Use of a Pseudomonas aeruginosa vaccine in patients with acute leukemia and cystic fibrosis. Amer. J. Med. 58: 629-636, 1975.

2. Reynolds, H.Y., Levine, A.S., Wood, R.E., Zierdt, C.H., Dale, D.C. and Pennington, J.E.: Pseudomonas aeruginosa infections: Persisting problems and current research to find new therapies. Ann. Intern. Med. 82: 819-831, 1975.

3. Levine, A.S.: Support systems for the patient with cancer - Future prospects. Cancer 36: 813-820, 1975.

4. Levine, A.S., Robinson, R. and Hauser, J.M.: Analysis of studies on protected environments and prophylactic antibiotics in adult acute leukemia. Europ. J. Cancer 11: 57-66, 1975

5. Elin, R., Robinson, R., Levine, A.S. and Wolff, S.: Lack of clinical usefulness of the limulus test in the diagnosis of endotoxemia. New Eng. J. Med. 293: 521-524, 1975.

6. Levine, A.S.: Protected environment - Prophylactic antibiotic programs: Clinical studies. Clinics in Hematology, in press.

7. Gill, F., Maclowry, J., Robinson, R. and Levine, A.S.: The relationship of fever, granulocytopenia and antimicrobial therapy to bacteremia in cancer patients. Cancer, in press.

8. Hande, K.R., Witebsky, F.G., Brown, M.S., Schulman, C.B., Levine, A.S., Anderson, S.E., Maclowry, J.D. and Chabner, B.A.: Sepsis with a new species of corynebacterium. Ann. Int. Med., in press.

9. Pizzo, P.A., Lovejoy, F.H., Jr. and Smith, D.: Prolonged fever in children: Review of 100 cases. Pediatrics 55: 468, 1975.

10. Alter, H., Chalmers, T., Freeman, B., Lunceford, J., Lewis, T., Holland, P.V., Pizzo, P.A., Plotz, P. and Meyer, W., III: Health care workers positive for hepatitis B surface antigen: Are their contacts at risk? New Eng. J. Med. 292: 454, 1975.

11. Pizzo, P.A.: Fever of unknown origin. In Hoekelman, R.A., Blatman, S., Brunell, P.A., Friedman, S.B. and Seidel, H.M. (Eds.): Principles of Pediatrics: Health Care of the Young. McGraw Hill, in press.

12. Poplack, D.G. and Jacobs, S.A.: Candida arthritis treated with amphotericin B. J. Pediat. 87: 989-990, 1975.

Contract Number: NO1-CM-23718

Contractor: University of Notre Dame, Notre Dame, Indiana

Principal Investigators: Morris Pollard, Ph.D. and Morris Wagner, Ph.D.

Budget: $116,258

Man Years: 7.8 (5.4 = professional, 2.4 = other)

Objective:

The principal objective of this project is to provide a better understanding of the interactions between microorganisms and the immunoincompetent leukemic host. More specifically, this study is directed toward determining whether germ-free animals or animals with a well-defined avirulent microflora can resist infections from either endogenous or exogenous sources.

Progress:

Conventional ADR mice were rendered germ-free by treatment with antibiotics and isolation in a germ-free environment. The animals continued to be germ-free when antibiotic treatment was stopped, so long as they remained in the germ-free isolators. Allogeneic or syngeneic bone marrow cells could be successfully transplanted in these animals. Germ-free animals repopulated with innocuous organisms ("Hexaflora") could partially resist challenges from exogenous pathogens.

Spider monkeys could not be permanently rendered germ-free.

Plan:

Contract has been terminated.

Publications:

1. Truitt, R.L., Pollard, M. and Srivastava, K.K.: Allogeneic bone marrow chimerism in germ-free mice. III. Therapy of leukemic AKR mice. Proc. Soc. Exp. Biol. Med. 146: 153-158, 1975.

2. Wagner, M. and Srivastava, K.K.: Decontamination of gnotobiotic mice experimentally monoassociated with Candida albicans. Infection and Immunity 12: 1401-1404, 1975.

3. Srivastava, K.K., Pollard, M. and Wagner, M.: Antileukemia therapy of antibiotic-decontaminated ADR mice. J. Infect. Dis., in press.

4. Srivastava, K.K. and Pollard, M.: Decontamination of conventional hamsters by antimicrobial agents. J. Lab. Animal Sciences, in press.

Appendix B

Contract Number: NO1-CM-53832

Contractor: M.D. Anderson Hospital and Tumor Institute, Houston

Principal Investigator: Gerald Bodey, M.D.

Budget: $550,000

Man Years: 10 (2 = professional, 8 = other)

Objective:

To investigate the utility of a protected environment-prophylactic antibiotic regimen in adult patients with non-Hodgkin's lymphoma or soft tissue sarcomas. The investigators wish to learn if this type of prophylaxis will reduce the incidence of infection in patients undergoing cancer chemotherapy, if a reduction in the incidence of infection will permit more intensive chemotherapy to be given, and if more intensive chemotherapy will produce a better overall anti-tumor response in terms of remission rate and duration.

Methods:

Patients are randomized in prospectively controlled studies to receive escalating cycles of chemotherapy in a protected environment (with comprehensive prophylactic anti-microbials) or in a conventional hospital setting. Each cycle of chemotherapy is escalated only if the previous cycle was not associated with infection.

Progress:

A total of 61 patients have been entered on these protected environment-prophylactic antibiotic studies. About one-half have had lymphoma and one-half sarcoma; half again have been in the protected environment (laminar-flow) and half controls.

For the lymphomas, the complete remission rate is 71% on the protected environment program, compared to 69% without protection. The median duration of response is 21+ weeks for protected environment patients, and 22+ weeks for controls. 7% of patients treated within the protected environment developed severe infection, compared to 25% without the protected environment. All patients in the protected environment were able to receive escalated dosage of chemotherapy, compared to 80% in the conventional setting.

For the sarcomas, the complete-plus-partial response rate is 72% in patients in the protected environment, compared to 44% in the conventional setting. Median survival is 72 weeks for patients in the protected environment and 40 weeks for patients in the conventional setting. 9% of patients in the protected environment developed severe infection, compared to 33% without the

protected environment. All patients in the protected environment had their dosage escalated, compared to 80% of the patients not in the protected environment.

Significance to Biomedical Research and the Program of the Institute:

Infection is the major cause of morbidity and mortality in patients with malignant disease. It is possible that if the incidence of infection could be reduced, more aggressive therapy might be tolerated and that this therapy would enhance remission and survival rates. Non-Hodgkin's lymphomas and sarcomas of adults may be responsive to such aggressive therapy. If so, prophylaxis of these patients from infection by a protected environment might provide an enduring therapeutic approach to these tumors.

Plan:

Since the remission rate for the lymphomas is comparably good for both protected environment patients and control patients, and since the median duration of response is also comparable in both settings, there is little to be gained from continuing the lymphoma study. On the other hand, the sarcoma study shows a trend toward improved response rates in the protected environment as opposed to the control setting. Therefore, the lymphomas study will be discontinued, and the sarcoma study will be continued with an attempt to make the tumor population as homogeneous as possible in terms of sarcoma type. A new study of breast carcinoma will be initated; the study design will be the same, i.e., escalating chemotherapy in or out of a protected environment.

Publications: None

Appendix C

Contract Number: NO1-CM-53831

Contractor: Children's Hospital of Los Angeles

Principal Investigator: Stuart Siegel, M.D.

Budget: $314,492

Man Years: 10 (2 = professional, 8 = other)

Objective:

To investigate the utility of a protected environment-prophylactic antibiotic regimen in children with sarcomas, neuroblastoma, or non-Hodgkin's lymphomas. The investigators wish to learn if this type of prophylaxis will reduce the incidence of infection in patients undergoing cancer chemotherapy, if a reduction in the incidence of infection will permit more intensive chemotherapy to be given, and if more intensive chemotherapy will produce a better overall anti-tumor response in terms of remission rate and duration.

Methods:

Patients are randomized in prospectively controlled studies to receive escalating cycles of chemotherapy in a protected environment (with comprehensive prophylactic anti-microbials) or in a conventional hospital setting. Each cycle of chemotherapy is escalated only if the previous cycle was not associated with infection.

Progress:

A prospectively controlled study has been instituted to determine whether the use of protected environments (PE) can reduce the risk of serious infection during intensive therapy of disseminated childhood solid tumors. Children between the ages of 6 months and 16 years with Stage IV soft tissue sarcomas, Stages III and IV neuroblastoma, and Stages II, III, and IV non-Hodgkin's lymphoma are randomized to therapy in either the PE or a normal treatment setting without antibiotic decontamination (control). Environmental surveillance cultures of the PE revealed 96% sterile air and 90% sterile surface samples. 89% of stool cultures in PE patients were sterile compared to 82% of skin cultures and 38% nasopharyngeal cultures. In contrast, organisms were regularly recovered from throat cultures. Fifteen patients have been entered on study, 8 in the PE group and 7 in the control group. The two groups are comparable as to diagnosis, severity and duration of granulocytopenia, and other risk factors. Patients in the PE have experienced 1.65 febrile episodes/100 patient days *vs.* 2.50 in the control group. Five serious infections have occurred in the control group compared to 2 in the PE group. One infectious death has occurred in the control group. A microbiologically effective protected environment program for the pediatric patient has been

developed without limiting toxicity or patient resistance.

Significance to Biomedical Research and the Program of the Institute:

Infection is the major cause of morbidity and mortality in patients with malignant disease. It is possible that if the incidence of infection could be reduced, more aggressive therapy might be tolerated and that this therapy would enhance remission and survival rates. Solid tumors of childhood may be responsive to such aggressive therapy. If so, prophylaxis of these patients from infection by a protected environment might provide an enduring therapeutic approach to these tumors.

Plan:

There is not yet sufficient data on tumor responsiveness in or out of the protected environment to form a statistical conclusion. The contract will be reviewed on a yearly basis.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CM-06840-01 PO

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Tumor Immunology and Immune Surveillance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. G. Leventhal	Head, Chemoimmunotherapy Section	PO C
	D. G. Poplack	Senior Investigator	PO C
OTHER:	D. L. Mann	Senior Investigator	I C
	R. M. Blaese	Head, Cellular Immunology Section	MET C

COOPERATING UNITS (if any)
Immunology Branch, Metabolism Branch (NCI)

LAB/BRANCH
Pediatric Oncology Branch

SECTION
Chemoimmunotherapy Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2

SUMMARY OF WORK (200 words or less - underline keywords)

In this project, the effects of chemotherapy and immunotherapy on the immune response to tumor and other antigens are assessed. Attempts are also made to detect, characterize and purify tumor antigens in acute leukemia. Studies also include the development of a clinical test of human monocyte function, the development of an assay of macrophage activation using colloidal $_{198}Au$, and the utilization of monocyte chemotaxis to assess the response to different immunostimulators. The assays of monocyte and macrophage function are applied to patients with immunodeficiency syndromes, including the Wiskott-Aldrich syndrome, and to patients with malignancies.

Project Description:

Objectives:

1) To assess the effect of chemotherapy and immunotherapy on the immune response to tumor and other antigens.

2) To detect, characterize and purify tumor antigens in acute leukemia.

Major Findings:

A. Serial immune evaluations are being performed on most patients in the Pediatric Oncology Branch. On protocol 72-1 (acute lymphatic leukemia induction, consolidation, and maintenance) all patients receiving BCG during maintenance developed a vigorous skin test response to PPD; however those patients who had received 5-day consolidation courses of Ara-C+6TG never developed a vigorous in vitro response to tuberculin. All other recall skin test responses remained stable throughout the study period, while in vitro responses to antigens and PHA showed a progressive decline with time. We interpreted this to mean that the recognition and memory functions of the immune system were intact, but that the proliferative response was impaired with chemotherapy, particularly after Ara-C+6TG consolidation.

B. In recent years, the macrophage-monocyte has been increasingly implicated as playing a central role in the host defense against both tumor and infection. We have studied the immunology of the monocyte-macrophage in both experimental animal systems and in man. Our current investigations include:

1. Development of a Clinical Test of Human Monocyte Function. We have developed a clinical assay of human monocyte function based on the ability of human monocytes to kill antibody-coated chromated target red cells. Using this cytotoxicity assay, we have been able to detect both quantitative and qualitative defects in monocyte function in patients with a number of disorders. We have discovered a defect in monocyte killer function in patients with the Wiskott-Aldrich Syndrome. These profoundly immunodeficient individuals are susceptible to chornic viral infections and develop lymphoreticular malignancies. This monocyte cytotoxic defect may play a central role in the pathogenesis of this disorder and its sequelae. We are currently investigating the cause of this defect and have expanded our studies of monocyte cytotoxicity to include patients with malignancies and other immunodeficiency diseases.

2. Development of an Assay of Macrophage Activation Using Colloidal ^{198}Au. We have developed an assay of macrophage activation which is based on the ability of macrophages to pinocytose selectively colloidal ^{198}Au. This assay has been used successfully in a guinea pig system (see below) and now is being adapted to evaluate human monocyte function.

3. Utilization of Monocyte Chemotaxis to Assess the Response to Different Immunostimulators. We have studied monocyte chemotaxis and macrophage colloidal ^{198}Au uptake in a guinea pig system. With this system, we have evaluated the effectiveness of various immunoadjuvants. We have found that different adjuvants stimulate the macrophage-monocyte to different degrees. For example, BCG, which stimulates colloidal ^{198}Au uptake, also stimulates random migration and chemotactic responsiveness. Other adjuvants studied (including C. Parvum, MER and Levamisole) also stimulate chemotactic responsiveness but do not effect random migration.

   We are currently studying patients receiving immunotherapy with similar techniques.

Significance to Biomedical Research and the Program of the Institute:

Our studies have revealed the antigenic complexity of the acute lymphocytic leukemia cell, and the relative lack of clinical efficacy of both specific and non-specific immunotherapy in acute lymphocytic leukemia. In recent years, however, the macrophage-monocyte system has been increasingly implicated as playing a central role in the host defense against both tumor and infection. The development of assays of human monocyte function and macrophage activation, as well as the utilization of monocyte chemotaxis to assess the response to immunostimulators, are of singular importance in permitting further understanding of the monocyte-macrophage system in human disease. It is hoped that the development of these assays will also provide a more rational basis for immunotherapy in such patients.

Proposed Course:

Current protocols for acute leukemia which utilize immunotherapy will continue until they reach statistical significance. The tests of monocyte and macrophage function and activation which have been developed will be applied to a wide variety of patients with immunodeficiency syndromes, in particular those syndromes which eventuate in the development of malignant disorders. These new laboratory assays will also be applied to many patients with primary malignant diseases in order to determine their role in explicating tumor mechanisms and in permitting rational development of immunotherapy regimens.

Publications:

1. Leventhal, B.G., Poplack, D.G., Johnson, G.E., Simon, R., Bowles, C. and Steinberg, S.: The effect of chemotherapy and immunotherapy on the response to mitogens in acute lymphatic leukemia. In Oppenhein, J. (Ed.): Role of Mitogens in Immunobiology. New York, Academic Press, in press.

2. Leventhal, B.G. and Konior, G.S.: Immunological treatment of neoplasms in man. In Green, I., Cohen, S. and McCluskey, R.T. (Eds.): Mechanisms of Tumor Immunity. J. Wiley and Sons, Inc., in press.

3. Poplack, D.G., Sher, N.A., Chaparas, S.D., Hirschberg, J. and Blaese, R.M.: The effect of BCG (mycobacterium bovis) on macrophage chemotaxis and colloidal ^{198}Au uptake in the guinea pig. Cancer Research, in press.

4. Gerety, R., Poplack, D.G., Hoofnagle, J.H., Barker, L.F. and Blaese, R.M.: Hepatitis B virus infection in the Wiskott-Aldrich syndrome. J. Pediat., in press.

5. Leventhal, B.G., Johnson, G.E., Bryan, J.H. and Halterman, R.H.: Speculation on the significance of the in vitro lymphocyte response to autologous tumor. In Workshop on Prognostic Factors in Human Acute Leukemia. In Fliedner, T.M. and Perry, S. (Eds.): Advances in the Biosciences. Pergamon Press, 1975, vol. 14, pp. 461-464.

6. Herberman, R.B., Char, D.H., Oldham, R.K. and Leventhal, B.G.: The prognostic value of studies of specific cell-mediated immunity in acute leukemia. In Workshop on Prognostic Factors in Human Acute Leukemia. In Fliedner, T.M. and Perry, S. (Eds.): Advances in the Biosciences. Pergamon Press, 1975, vol. 14, pp. 431-440.

7. Mann, D.L., Leventhal, B.G. and Halterman, R.: Human antisera detecting leukemia-associated antigens on autochthonous tumor cells. Journal of the National Cancer Institute 54: 345-347, 1975.

8. Leventhal, B.G.: Immune status in acute leukemia. In Sinks, L.F. and Godden, J.O. (Eds.): Conflicts in Childhood Cancer. An Evaluation of Current Management. New York, Alan R. Liss, Inc., 1975, vol. 4.

9. Mann, D.L., Halterman, R. and Leventhal, B.G.: Leukemia associated antigens detected with serums from immunized patients. Cancer Research, in press.

10. Poplack, D.G. and Blaese, R.M.: The mononuclear phagocyte system. In Stiehm, E. and Fulginetti, V. (Eds.): Immunologic Disorders in Infants and Children, in press.

11. Blaese, R.M. and Poplack, D.G.: Disorders of the mononuclear phagocytic system. In Stiehm, E. and Fulginetti, V. (Eds.): Immunologic Disorders in Infants and Children, in press.

Appendix A

Contract Number: NO1-CM-23701

Contractor: Hazleton Laboratories of America, Inc.

Principal Investigator: Charles A. Bowles, Ph.D.

Budget: $126,760

Man Years: 2

Objective:

This contract has essentially been set up as a service contract to aid in the intensive immunologic investigations being pursued in the Pediatric Oncology Branch.

Methods:

Measurements of lymphocyte responses to in vitro stimulation with mitogens, antigens and allogeneic cells have been performed in three different groups of subjects:

1. Lymphocyte responses by cells from leukemic patients before, during and after chemotherapy and/or combination chemotherapy-immunotherapy. Stimulants include the above mitogens and antigens, irradiated autologous and allogeneic cells, RAJI cells and autologous and allogeneic tumor cells.

2. Mixed leukocyte reactions are done with selected leukemia patients prior to marrow transplantation.

3. Another major effort involves similar tests in normal and lymphosarcoma-bearing dogs that are receiving chemotherapy and/or marrow transplants.

Significance to Biomedical Research and the Program of the Institute:

This contract provides for the immunologic evaluation of patients receiving immunotherapy, and related research investigations. The main purpose of these studies is to evaluate the utility of immunotherapy in the treatment of acute leukemia.

Plan:

Contract to be reviewed on yearly basis.

Publications:

1. Bowles, C.A., White, G.S. and Lucas, D.J.: Rosette formation by canine peripheral blood lymphocytes. Immunol. 114: 399-402, 1975.

Appendix B

Contract Number: NO1-CM-12127

Contractor: Hebrew University, Jerusalem, Israel

Principal Investigators: Dr. David Weiss and Dr. Gaby Izak

Budget: $155,424

Man Years: 3

Objective:

To investigate the therapeutic effect and the effect on the immune response of MER (methanol-extraction-residue of BCG) in tumor models and in patients with AML.

Methods:

1. A variety of immune parameters in a number of animal models (including mouse leukemia and guinea pig hepatomas) are being studied to assess the role and mechanism of stimulatory action of MER upon the immune response.

2. A clinical study of the therapeutic efficacy of MER as an adjuvant to maintenance therapy in acute myelogenous leukemia.

Findings:

Thus far 35 AML patients have entered the study. The data to date suggests that both remission duration and survival are longer in the group that received MER than in the group receiving chemotherapy alone. Immunologic assessment of patients in both groups has shown that the MER patients have heightened T cell immunoresponsiveness.

Significance to Biomedical Research and the Program of the Institute:

The animal studies in this project constitute a significant contribution to our understanding of the mechanism of action of immuno-adjuvants (in this case MER) at the cellular level. The clinical trial is yielding suggestive evidence for some therapeutic efficacy of MER as an adjunct to maintenance chemotherapy in patients with AML.

Plan:

The study will continue to accrue patients. The contract will be carefully monitored and reviewed on a yearly basis.

Publications:

1. Gallili, N., Naor, D., Asjo, B. and Klein, G.: Induction of immune responsiveness in genetically low-responsive tumor host combination by chemical modification in the immunogen, in press.

2. Weiss, D.W., Kuperman, O., Fathallah, N. and Kedar, E.: Mode of action of mycobacterial fractions in anti-tumor immunity: Preliminary evidence for a direct nonspecific stimulatory effect of MER on immunologically reactive cells. Ann. N.Y. Acad. Sci., in press.

3. Wainberg, M.A., Minden, P., Margolese, R.G. and Weiss, D.W.: Differential responsiveness of various substrains of inbred strain 2 guinea pigs to immunotherapy with the methanol extraction residue (MER) of BCG, in press.

4. Steinitz, M. and Weiss, D.W.: Studies on the physiological manifestations of cell-mediated cytotoxicity. I. Early metabolic changes in mouse plasmacytoma cells exposed in vitro to densitized allogeneic splenocytes. Cell. Immunol. 15: 403-418, 1975.

5. Steinitz, M., Feigis, M. and Weiss, D.W.: Studies on the physiological manifestations of cell mediated cytotoxicity. II. Inhibition of H^3 thymidine incorporation by plasmacytoma cells exposed in vitro to sensitized splenocytes. Cell. Immunol. 17: 181-192, 1975.

6. Doljanski, F., Markson, Y., Wainberg, M.A. and Weiss, D.W.: Cellular immunity to Rous sarcoma (RS) - Preferential autochthonous interactions; and, application of the immunoadherence technique to mouse tumors. Transpl. Proc. 7: 519-523, 1975.

7. Doljanski, F., Markson, Y., Katsav, S. and Weiss, D.W.: Studies on the initial phases of effector cell-tumor target cell interactions. Rous sarcomas (RS) of chickens. Proc. Fifth International Conference on Lymphatic Tissue and Germinal Centers in Immune Reactions, Tiberias, June 24-30, 1975, in press.

8. Doljanski, F., Markson, Y., Katsav, S. and Weiss, D.W.: Studies on the initial phases of effector cell-tumor interactions. Rous sarcomas (RS) of chickens. Proc. Fifth International Conference on Lymphatic Tissue and Germinal Centers in Immune Reactions, Tiberias, June 24-30, 1975, in press.

9. Stupp, Y., Saltoun, R. and Weiss, D.W.: Prevention by the MER tubercle bacillus fraction of immunosuppression induced by cancer chemotherapeutic agents. I. Antibody response of mice treated with cyclophosphamide, in press.

10. Pass, E., Jacobs, D.M. and Weiss, D.W.: Modulation of the immune response by the methanol insoluble fraction (MER) of attenuated tubercle bacilli. III. Stimulation of antibody synthesis to a soluble protein, BGG, in press.

11. Ben-Effraim, S. and Diamantstein, T.: Mitogenic and adjuvant activity of a methanol extraction residue (MER) of tubercle bacilli on mouse lymphoid cells in vitro. Immunol. Communic. , in press.

12. Ben-Efraim, S., Ulmer, A., Schmidt, M. and Diamantstein, T.: Differences between lymphoid cell populations of guinea pigs and mice as determined by the response to mitogens in vitro. Int. Arch. Allergy , in press.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06850-06 PO

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

DNA and RNA Tumor Viruses: Molecular Mechanisms of Infection and Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I:	A.S. Levine	Head, Infectious Diseases Sect.	PO C
OTHER:	C.T. Patch	Senior Investigator	PO C
	S.K. Chattopadhyay	Senior Staff Fellow	PO C
	I.T. Magrath	Visiting Scientist	PO C
	P.A. Pizzo	Senior Investigator	PO C
	G.W. Jay	Cancer Research Expert	PO C
	E. Everitt	Visiting Fellow	PO C
	M.W. Oster	Clinical Associate	PO C
	A.M. Lewis	Senior Investigator	LVD I
	W.P. Rowe	Chief, Lab. of Viral Diseases	LVD I
	P. Sharp	Assistant Professor	MIT
	N. Davidson	Professor	CAL Tech
	F. Ruddle	Professor	Yale Univ.
	J. Wrathall	Assistant Professor	Georgetown

COOPERATING UNITS (if any) Laboratory of Pathology (NCI); Laboratory of Viral Diseases (NIAID); Center for Cancer Research (MIT); Division of Human Genetics, Yale; Biochemistry Department (CAL Tech.); Department of Anatomy, Georgetown; Laboratory of Biology of Viruses (NIAID)

LAB/BRANCH
Pediatric Oncology Branch

SECTION
Infectious Disease Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
15	10	5

SUMMARY OF WORK (200 words or less - underline keywords)

We are studying the genetic organization and expression of SV40 and adenovirus 2 by an analysis of the SV40 and Ad2 genomes contained within Ad2-SV40 hybrid virus DNA molecules. The hybrid viruses are also employed as models for understanding the behavior of integrated genomes. We are also analyzing the behavior of SV40 and Ad2-transformed or lytically infected cells as a function of the molecular conformation of viral nucleic acids within these cells, and as a function of events which may control viral replication, transcription, and translation. Secondly, we are analyzing the state and expression of the endogenous AKR murine leukemia virus genome in mice with varying expression of leukemia and virus production. A further objective is to explore the evolution, transmission, and relation to leukemogenesis of various murine RNA viruses. Finally, this laboratory is concerned with production and purification of Epstein-Barr virus DNA and the use of this reagent to explore molecular mechanisms of infection and oncogenicity associated with this human herpes virus.

Project Description:

Objectives:

1) To study the genetic organization and expression of Simian Virus 40 (SV40) and human Adenovirus 2 (Ad2) by an analysis of the varying segments of the SV40 and Ad2 genomes contained within Ad2-SV40 hybrid virus DNA molecules. Such studies employ biologic, biochemical, biophysical, and electron microscopic techniques to achieve genetic maps of these animal tumor viruses. The hybrid viruses are also employed as models for understanding the behavior of integrated genomes, the phenomenon of integration appearing central to the molecular mechanism of viral oncogenesis.

2) To analyze the behavior of SV40 and Ad2-transformed or lytically infected cells as a function of the molecular conformation of viral nucleic acids within these cells, and as a function of events which may control viral replication, transcription, translation, and maturation.

3) To improve the production and purification of Epstein-Barr virus (EBV) DNA and to use the reagent to explore the molecular mechanisms of infection and oncogenicity associated with this human herpes virus.

4) To analyze the state and expression of the endogenous AKR murine leukemia virus genome in rodents with high-, low-, or no incidence of leukemia. A further objective is to explore the evolution, transmission and relation to leukemogenesis of this virus, using cells from a variety of rodents and related species indigenous to widespread parts of the world.

Methods Employed:

Procedures for the isolation of highly purified viral and cell macromolecules are continuously improved and new techniques for hybridization of viral and cellular nucleic acids are developed. A variety of techniques which permit quantitative comparison of nucleotide sequences are in use.

This laboratory routinely employs equilibrium density centrifugation, analytical ultracentrifugation, electron microscopic heteroduplex mapping, enzymatic fragmentation and strand separation of viral DNA, gel electrophoresis, hydroxyapatite chromatography, and the in vitro synthesis of viral DNA probes. Nucleotide sequencing of specific viral RNA transcripts is available. In vitro protein synthesis using cell-free systems is undertaken. An extensive tissue culture facility is maintained.

Mouse breeding experiments are performed in association with studies on the genetics of RNA tumor viruses. Radiolabelled proteins are analyzed via autoradiography or fluorography; fingerprint analysis of the tryptic peptides of purified proteins is performed by a two-dimensional procedure involving high-voltage eletrophoresis and thin-layer chromotography.

Major Findings:

A. Studies of Naturally Occurring Murine Leukemia Viruses

Utilizing a viral DNA probe synthesized from AKR leukemia virus RNA, we have established that a good correlation exists between the completeness of the viral sequences in the host genome, the number of copies of the viral-specific sequences, leukemogenesis, and the production of virus by various mouse strains. Furthermore, we have demonstrated that a major virus-inducing locus, Akv-1, is a viral structural gene. We have extended these observations by analyzing various other inbred mouse strains and feral mus musculus from different parts of the world. We have also analyzed several species of the genera mus and mus-related genera. A picture of the evolution of these naturally occurring murine leukemia viruses is now emerging.

Halogenated pyrimidines (IUDR and BUDR) activate C-type virus production from normal cells of a wide variety of animals in tissue culture. The pyrimidines become incorporated into the DNA of these cells, but the mechanism by which they activate virus is not known. In a normal (non-producer) cell line derived from AKR embryos, about 30-40 percent of the viral sequences are present in cellular RNA. However, after treatment with IUDR, 100 percent of the viral-specific sequences are expressed in cell RNA. In an attempt to understand the mechanism of this IUDR effect, we wished to learn whether new viral-specific sequences are exclusively transcribed after IUDR treatment, or whether this drug increases the overall transcription of cellular DNA with integrated viral sequences comprising one part. Preliminary hybridization experiments suggest that IUDR activates unique-sequence mouse DNA transcription generally.

With Dr. Norman Davidson, we have examined the structure of genomic RNA of certain RNA tumor viruses. In these experiments, short lengths of poly dT are polymerized onto nicked circular SV40 DNA; the RNA tumor virus molecules of interest are then hybridized to the poly dT circles and the complexes are examined by electronmicroscopy. (Davidson has found that several oncornaviruses, e.g., RD114 and woolly monkey virus, contain a dimer of RNA subunits held together in a central secondary structure - the dimer linkage.) We have now looked at the mouse-tropic AKR-type murine leukemia virus RNA (naturally occurring), and this virus has a similar dimeric structure with poly A at both ends. Observations on xenotropic and amphotropic viral RNAs are in progress.

With Dr. Frank Ruddle, we are attempting to assign chromosomal locations for mouse-tropic and xenotropic viral genomes. Ruddle's group has developed somatic cell hybrids between cells from the "A" strain of mice and Chinese hamsters. In these hybrids, mouse chromosomes are preferentially lost. On the other hand, hamster cells contain about 20 percent of very poorly matched AKR viral sequences. Thus with various mouse chromosomes on a hamster chromosome background, we should be able to assign chromosomal loci for the mouse-tropic and xenotropic viruses.

B. Simian Virus 40 DNA Replication, Transcription, and Antigen Induction During Infection with Two Adenovirus 2-SV40 Hybrids that Contain the Entire SV40 Genome

The Ad2++HEY hybrid virus produces SV40 efficiently during lytic infection, whereas Ad2++LEY does not, although both hybrids contain a complete SV40 genome. We have demonstrated the synthesis of nonhybrid SV40 DNA in Ad2++HEY-infected cells but not in Ad2++LEY-infected cells. Hybridization-competition experiments indicate that both early and late SV40 RNA is transcribed efficiently in Ad2++HEY-infected Vero cells, but only early SV40 RNA is transcribed efficiently in Ad2++LEY-infected cells. Ad2++HEY induces SV40 U, T, and V antigens during lytic infection of African green monkey kidney cells, whereas Ad2++LEY induces only SV40 U and T antigens. These variations in the behavior of Ad2++HEY and Ad2++LEY regarding expression of SV40 functions probably reflect differences in the rate of SV40 excision from the hybrid genomes.

C. Adenovirus Transcription. II. RNA Sequences Complementary to Simian Virus 40 and Adenovirus 2 DNA in Ad2+ND_1-and Ad2+ND_3-Infected Cells

The genomes of the two nondefective Ad2/SV40 hybrid viruses, Ad2+ND_1 and Ad2+ND_3, were formed by a deletion of about 5% of Ad2 DNA and insertion of part of the SV40 genome. We have compared the cytoplasmic RNA synthesized during both the early and late stages of lytic infection of human cells by these hybrid viruses to that expressed in Ad2-infected and SV40-infected cells. The fraction of each fragment of Ad2 DNA expressed as cytoplasmic RNA was determined by annealing fragmented ^{32}P-labeled Ad2 DNA strands to an excess of cellular RNA extracted from infected cells. The segment of Ad2 DNA deleted from both hybrid virus genomes is transcribed into cytoplasmic mRNA during the early phases of Ad2 infection. Hence, we suggest that Ad2 codes for at least one "early" gene product which is nonessential for virus growth in cell culture. In both early Ad2+ND_1 and Ad2+ND_3-infected cells, 1,000 bases of Ad2 DNA adjacent to the integrated SV40 sequences are expressed as cytoplasmic RNA but are not similarly expressed in early Ad2-infected cells. The 3' termini of this early hybrid virus RNA maps in the vicinity of 0.18 on the conventional SV40 map and probably terminates at the same position as early lytic SV40 cytoplasmic RNA. Therefore, the base sequence in this region of SV40 DNA specifies the 3' termini of early messenger RNA present in both hybrid virus and SV40-infected cells.

D. The Content and Expression of Integrated Viral DNA in Hamster Cells Transformed by the Nondefective Adenovirus 2-Simian Virus 40 Hybrid Viruses

Hamster cells transformed by Ad2 and five nondefective Ad2-SV40 hybrid viruses are all of the Ad2-transformed phenotype. All lines accumulate Ad2 RNA and Ad2 T antigen; two hybrid-transformed lines accumulate SV40 RNA but only one contains detectable amounts of SV40 antigens. We examined selected lines from this group of transformed hamster cells for Ad2 and SV40 DNA content and RNA expression using separated strands of

labeled viral DNA as probes and hydroxyapatite chromatography. All lines studied contain multiple copies of incomplete Ad2 genomes, and they may or may not contain SV40 DNA. In the lines containing SV40 DNA, the entire SV40 (-) strand is transcribed; the (+) strand is not expressed in these cells. The expression of Ad2 RNA exhibits two distinct patterns. In one pattern, transcription of the heavy strand of Ad2 DNA is less extensive than that of the light strand, while in the second pattern of Ad2 RNA expression, the extent of heavy strand transcription is greatly increased. These patterns of viral RNA expression suggest that host promoters play an important role in regulating transcription of integrated viral DNA. The presence of SV40 DNA in the hybrid viral genome may influence the selection of cellular DNA integration sites or the orientation of the viral genome at the site of integration.

E. Fate of the Precursor to the Major Core Protein During Adenovirus Assembly

An isopycnic Metrizamide-detergent density gradient system was developed in which the newly synthesized precursor (polypeptide P-VII) to the major core protein of adenovirus type 2 (polypeptide VII) was confined to a spectrum of complexes with densities equal to or greater than that of adenovirus. The radioactivity of polypeptide P-VII within the region of protein density was readily chased into polypeptide VII under *in vivo* conditions. No transition of radioactivity was detectable after *in vitro* incubation. However, newly synthesized P-VII present at a density region intermediate between virion and protein densities was readily processed *in vitro* at 4C. No structures equivalent to the core structure of disrupted virions or to incomplete particles were detected in this system.

F. Identification, Purification, and Localization of Early SV40 Antigens

Using 35S-labeled methionine, we have been able to label virus-specific polypeptides at very high specific activity and to identify on SDS-polyacrylamide gels the SV40-specific polypeptides coded for by the various adeno 2-SV40 hybrid viruses. SV40 polypeptides of different molecular weights (28K, 42-56K) have been detected in cells infected by Ad2+ND_1 and Ad2+ND_2. Both of these viruses induce SV40 U antigen, and both possess the early SV40 function which enhances the growth of human Ad2 in otherwise nonpermissive monkey cells. We have purified the two polypeptides of different weight from ND_1 and ND_2-infected cells and are comparing their tryptic peptide fingerprints to determine whether the smaller 28K protein found in ND_1-infected cells is a subset of the larger 42K or 56K protein found in ND_2-infected cells. This will show whether the 28K protein is actually a "functional deletion mutant" of a larger wild-type protein. We have been able to localize the 42K protein on 40S ribosomal subunits in the supernatant. 28K protein is not in the supernatant, but is enriched in the outer nuclear membrane fraction.

G. Expression of Integrated Viral Genomes

We have isolated from SV40-infected cells "transcriptional complexes" containing nascent RNA molecules associated with host cell DNA. The DNA

and nascent RNA purified from these complexes have been analyzed for the presence of SV40-specific sequences by hybridization to separated strands of SV40 DNA. We have found that integrated SV40 sequences, found during lytic infection of permissive cells, are transcriptionally inactive and that early SV40 RNA is not transcribed from these integrated viral DNA templates.

H. Purification and Characterization of EBV DNA

In these experiments, the HR1 cell line is used as a primary source of EBV. The cells are exposed to corticosteroids on varying schedules and grown at different temperatures. Virus yield is assessed by electron-microscopy of cells, immunofluorescent quantitation of late viral antigen, and production of virus from cytoplasm and culture supernatant. Virus purification is carried out using an initial velocity gradient and a second isopycnic banding. DNA purification is carried out by detergent lysis of virions and extraction with phenol and alcohols, or by sucrose gradients. We have found that corticosteroids markedly stimulate the production of EBV from HR1 cells grown at low temperature (32° centigrade). This has been verified by all of the parameters mentioned above. The viral DNA has been obtained in purified form with sedimentation characteristics of intact 10^8 dalton molecules.

Significance to Biomedical Research and the Program of the Institute:

In studies on the mouse-tropic, xenotropic, and amphotropic murine leukemia viruses, our objective has been to correlate specific leukemia virus sequences with specific biological events, especially leukemogenesis. Comparative study of the genetic information specified by different murine leukemia virus sequences in different strains of mice is feasible, and we believe that it will be possible to determine the evolutionary origin and transmission of murine leukemia virus infection. Our studies during the past year have explained why only some mice produce virus and develop leukemia even though all mice may contain MLV antigens. Moreover, it has been established that a major mouse chromosomal locus for the production of MLV is in fact composed of MLV nucleotide sequences, and is not merely a host locus which permits the expression of remote viral functions. It should be emphasized that these studies concern a known naturally occurring animal leukemia virus within the context of known and controlled host genetics. We believe that these studies will guide the framing of testable hypotheses in human leukemia, wherein a proven leukemia virus has not been characterized and where the genetics of the disease are not known and cannot be controlled.

Our studies of Ad2-SV40 hybrids have made a significant contribution to knowledge of the genetic organization and expression of the two tumor viruses during lytic infection and oncogenesis. Moreover, these hybrid virus studies offer insight into the state and expression of integrated heterologous DNA in general and must be considered in planning the type of "genetic engineering" experiments possible with recombinant DNA technology.

Whether a specific viral infection will lead to virus production or to cell transformation depends entirely upon the interaction between the incoming virus and the host cell involved. At the molecular level, such interactions may occur between virus-specific macromolecules and macromolecules specified by the host. Our studies with Ad2 and SV40 antigens are directed toward an understanding of these interactions; our hope is to provide a better understanding of the mechanisms of viral infection and oncogenesis.

EBV is closely associated with Burkitt's lymphoma and nasopharyngeal carcinoma, and probably causes infectious mononucleosis. Studies of the biology of the virus and the significance of its association with human malignant disease have been hampered by the miniscule quantities of virus obtained until now from even the best producer lines. Our greatly improved production of virus will permit much more sophisticated studies of both the biology and the biochemistry of this virus. These studies are designed to clarify as far as possible the nature of the association of EBV with human malignant disease.

Proposed Course:

We shall employ various biochemical and biological techniques to distinguish between the viral sequences specified by different endogenous murine leukemia viruses in the DNA of mice with differing expression of MLV information. DNA from other rodent species will be tested for homology with various MLV DNA probes, and these studies should speak to the evolutionary origin, transmission, completeness and expression of the MLV genome. We shall attempt to delineate the molecular mechanism of viral activation by halogenated pyrimidines, and will also extend these studies with other activating agents. Using mouse-hamster somatic cell hybrids, we shall try to localize other viral genes at the chromosomal level.

Studies on the nondefective Ad2-SV40 hybrid viruses will emphasize the kinetics of SV40 transcription early and late in infection. We shall also employ specific SV40 transcripts in in vitro protein-synthesizing systems to generate tumor-associated proteins. We shall continue our studies on the effects of SV40 insertion and Ad2 deletion on the regulation of expression of integrated DNA. In our studies on SV40 early antigens, we shall search for any biochemical change that may accompany the appearance of a specific viral antigen upon virus infection. With data obtained from our studies on the localization of SV40 antigens, we shall design cell-free systems to probe the function of specific antigens. Following completion of our studies on the expression of integrated SV40 genomes, i.e., "transcriptional complexes," we shall extend our findings to other viral systems, in particular infection of human KB cells with adenovirus 2.

In our experiments with the Epstein-Barr virus, we shall attempt to optimize the system for production and purification of this virus and its DNA: then we hope to measure the genetic relatedness of EBV strains of different origin, to study the relationship between EBV DNA and host cell DNA, and to relate our findings to EBV-associated human disease.

Publications:

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2. Lewis, A.M., Jr., Breeden, J.H., Wewerka, Y.L., Schnipper, L.E. and Levine, A.S.: Studies of hamster cells transformed by adenovirus 2 and the nondefective adenovirus 2-SV40 hybrids. Cold Spring Harbor Symposium on Quantitative Biology 39: 651-656, 1975.

3. Kelly, T.J., Jr., Lewis, A.M., Jr., Levine, A.S. and Siegel, S.E.: Structural studies on two adenovirus 2-SV40 hybrids which contain the entire SV40 genome. Cold Spring Harbor Symposium on Quantitative Biology 39: 409-417, 1975.

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5. Flint, S.J., Wewerka-Lutz, Y., Levine, A.S., Sambrook, J. and Sharp, P.A.: Adenovirus transcription. II. RNA sequences complementary to SV40 and adenovirus 2 DNA in $Ad2+ND_1$ and $Ad2+ND_3$-infected cells. J. Virol. 16: 662-673, 1975.

6. Dhar, R., Subramanian, K.N., Zain, B.S., Levine, A.S., Patch, C.T. and Weissman, S.M.: Sequences in SV40 DNA corresponding to the "ends" of cytoplasmic mRNA. In Haenni, A. and Beaud, G. (Eds.): In Vitro Transcription and Translation of Viral Genomes. Editions Inserm 47: 25-32, 1975.

7. Breeden, J.M., Wewerka-Lutz, Y., Schnipper, L.E., Hauser, J., Patch, C.T., Lewis, A.M., Jr. and Levine, A.S.: The content and expression of integrated viral DNA in hamster cells transformed by the nondefective adenovirus 2-simian virus 40 hybrid viruses. J. Virol., in press.

8. Everitt, E., Meador, S.A. and Levine, A.S.: Fate of the precursor to the major core protein during adenovirus assembly. J. Virol., in press.

9. Chattopadhyay, S.K., Rowe, W.P., Teich, N.M. and Lowy, D.R.: Definitive evidence that the murine C-type virus-inducing locus AKV-1 is viral genetic material. Proc. Nat. Acad. Sci U.S.A. 71: 906-910, 1975.

10. Lowy, D.R., Chattopadhyay, S.K. and Teich, N.M.: The chromosomal localization of an endogenous murine leukemia viral genome in the AKR mouse. J. Cell Physiol., in press.

11. Jay, G. and Kaempfer, R.: Translational repression of a viral messenger RNA by a host protein. J. Biol. Chem. 250: 5742-5748, 1975.

12. Jay, G. and Kaempfer, R.: Initiation of protein synthesis: The binding of messenger RNA. J. Biol. Chem. 250: 5749-5755, 1975.

13. Lavelle, G., Patch, C., Khoury, G. and Rose, J.: Isolation and partial characterization of single-stranded adenoviral DNA produced during synthesis of adenovirus type 2 DNA. J. Virol. 16: 775-782, 1975.

ANNUAL REPORT OF THE RADIATION ONCOLOGY BRANCH

NATIONAL CANCER INSTITUTE

July 1, 1975 through June 30, 1976

The past year has been highlighted in major part by the clinical investigations of the Branch. As mentioned in the Summary Report of last year, the emphasis of these clinical studies has been concentrated on efforts to improve the management of various malignancies using combined modality approaches. In the interim, there has continued an updating of previous clinical trials to which accession of patients has been terminated and the initiation of a major program designed to facilitate radiation treatment planning using computerized tomography, computerized dosimetry, and eventual implementation of computer-assisted delivery of radiation therapy.

The provocative experience using total body irradiation for the treatment of patients with active, progressive chronic lymphocytic leukemia has been extended. Review of a decade of experience had shown that one-third of patients experienced a remission status which extrapolated into not only prolonged survival but improved quality of life. More recent investigation which combines total body irradiation with chemotherapy has increased the remission rate to approximately 50-60% and will hopefully result in a substantial increase in the median survival for patients with this disease.

Meticulous long-term observation has been maintained on patients with Hodgkin's disease treated between 1965 and 1969. An advantage in survival has continued for those patients given extensive prophylactic irradiation and despite the aggressiveness of treatment, no deleterious hematologic or immunologic effects have been observed. In contrast to Hodgkin's disease where treatment-induced remissions have become reflected in long-term, disease free survival, the situation has been distinctly different in the non-Hodgkin's lymphomas. While considerable insight has been gained as to the biology and natural history of the lymphomas through careful staging studies and clinical observation, durable unmaintained remissions have been noted in only a minority of patients. Despite the ability to induce complete remissions using a variety of treatment methods, relapse has developed in approximately 70% of patients within five years with non-Hodgkin's lymphomas.

The program on Ewing's sarcoma, which has yielded survival data as yet unparalleled by other centers, has served to provide information on means of combining radiotherapy with combination chemotherapy as noted in the last Summary Report. This experience has served an essential purpose in

the initiation of and, to date, encouraging program for the treatment of small cell carcinoma of the lung. Using an intensive chemotherapeutic regime integrated with regional radiotherapy and central nervous system prophylaxis, a complete remission as determined by restaging evaluation was achieved in 20 of the first 21 consecutive patients with small cell cancer of the lung. This study was not restricted to ambulatory patients, some patients being bed-ridden upon admission and over one-half of the cases having clinical extrathoracic dissemination. While it is evident that the median survival exceeds one year, the more important observation is the unmaintained remission rate of 50% at one year in this preliminary experience.

A major project has been initiated during the past year to improve the quality of radiation treatment planning based on new technology involving computerized tomography and computerized dosimetry treatment planning. A whole-body computerized tomography (EMI) unit will be installed within the next month and auxillary computer equipment has been purchased. The thrust of this program is to utilize the high resolution imaging obtainable with computerized tomography to localize tumor volumes with greater accuracy, to take advantage of the attenuation coefficient (density) information inherent in the scanning process for correction of tissue inhomogeneities in dosimetry planning, to develop methods for treatment planning in three-dimensions, and finally to employ this information for the implementation of computer controlled administration of radiotherapy itself via interfacing of the radiotherapy units with a dedicated computer programed to monitor and control the various machine parameters.

Laboratory research has gradually expanded during the past year with particular emphasis on development of the capability for monitoring the immune mechanism of patients prior to, during, and following completion of treatment. This effort will be described in much greater detail in the Annual Report for the coming year.

The Radiation Oncology Branch has continued to provide ever increasing consultative and collaborative support to other intramural programs as well as provide non-clinical radiation exposures of biological materials and animals on a large scale basis. There were over 300 patients seen in formal consultation and an additional 200 logged telephone consultations provided "ad hoc" advice on treatment or general information. Although not included in the individual research project reports, there currently are collaborative protocols with other units involving soft tissue sarcomas, several pediatric malignancies, cancer of the pancreas, cancer of the ovary, and Hodgkin's disease.

Finally, comment is deserving on the considerable difficulty which steadily increases in terms of staffing the Branch, particularly with radiotherapists in view of the very competitive salaries offered at most universities. Unless the restrictions on government salaries are relieved within the next few years, it can be stated with relative assurance that serious difficulty will be encountered in maintaining staff adequate to continue a productive research program within the Radiation Oncology Branch.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CM 00650-21 R

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Service Radiation Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. C. Pomeroy, M.D.	Head, Rad. Ther. Sec.	R	NCI
OTHER:	C. H. Kent, M.D.	Senior Investigator	R	NCI
	R. N. Fairey, M.D.	Expert Consultant	R	NCI
	R. S. Swain, B.S.	Research Physicist	R	NCI
	L. R. Kirkland, Ph.D.	Research Physicist	R	NCI
	M. K. Glover, R.N.	Clinical Nurse	R	NCI
	M. R. Powell, R.T.	Medical Radiation Tech.	R	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	1.5	2.5

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to provide expert radiotherapy consultation and radiation treatment for Clinical Center patients admitted to services other than the Radiation Oncology Branch of the National Cancer Institute.

PHS-6040
(12-75)

Project Description:

Objectives: To provide facilities and services as required for the irradiation of animals and biological materials for research study by investigators at the National Institutes of Health. This includes consultation as to methods and types of irradiation and the preparation of material.

Methods Employed: A dual 250 Kvcp x-ray generator is provided, output routinely calibrated, and maintained in proper operating condition.

Major Findings: The attached list indicates the organizational use of the irradiation facilities. About 67% of the total exposures were made for investigators in the National Cancer Institute. 145 individuals made use of the irradiation facilities during the past year.

Significance to Biomedical Research and the Program of the Institute: Same as Major Findings.

Proposed Course: A new x-ray generator has been installed and is in operating condition at reduced output due to failure to receive one of the two x-ray tubes. It is expected to be fully operational by the end of April.

It is again recommended that consideration be given to the purchase of a self-contained cesium irradiator at a cost of about $60,000. 60% of the present users of the x-ray equipment have indicated a desire for this equipment. Since this equipment is not electronic in nature, more uniform dosimetry would result. Maintenance of this type of equipment is minimal and would reduce the maintenance problems of the x-ray equipment.

Publications: None except as the use of these facilities are indicated in publications by the various investigators.

Services Provided Using the Dual 250KVCP
Dual Unit X-ray Equipment
from April 1, 1975 to April 1, 1976

Organization	No. of Exposures
A-A&R	6
A-LCI	4
A-LI	192
A-LMB	64
A-LMI	84
A-LPB	25
BB-DBBP	132
BB-DV	189
C-BGY	84
C-CI	12
C-CMT	5
C-D	11
C-EXP	17
C-I	52
C-ID	88
C-LB	20
C-LCBGY	93
C-LEC	48
C-LMPH	140
C-LP	4
C-LPHY	1349
C-LTCB	13
C-M	149
C-RO	132
C-SURG	119
C-VB	186
C-VC	50
C-VLL	73
C-VO	5
CH-BB	5
CH-LMG	14
D-LBDA	50
D-LM	38
D-LMI	174
D-OMS	10
DRS-DIR	118
I-A&R	8
I-LCI	30
I-LI	67
I-LMI	100
I-LPD	42
I-LVD	54
M-LMC	10
N-LEN	6

Organization	No. of Exposures
*Bionetics	112
*Meloy	59
*Microbiological	9

*Exposures for Litton Bionetics, Meloy and Microbiological Associates Laboratories were performed on materials for projects under contracts from the National Cancer Institute.

Materials irradiated included cells, tissue culture, blood, parasites, mosquitoes, mice, rats, hamsters, chickens, guinea pigs and monkeys. Exposures ranged from 25R to 20,000R.

It is estimated that as many as 25% of exposures made were not recorded.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 00963-06 R

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunologic Manipulation of Radiation and Chemical Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. C. Pomeroy, M.D.	Head, Rad. Ther. Sec.	R	NCI
OTHER:	J. D. Lamberg, M.D.	Clinical Associate	R	NCI
	W. G. Hardy, M.D.	Biologist	R	NCI

COOPERATING UNITS (if any)

Laboratory of Pathology, DCBD, NCI

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to delineate the difference in neoplastic development of the R E tissues in comparison to epithelial and connective tissues by the contrasting effects of immune suppression and stimulation, and the effect of age on tumor incidence and patterns of neoplastic diversity.

Project Description:

Objectives: To evaluate the effects of age and immunologic manipulation on the incidence and time of appearance of radiation and chemical induction of neoplasms in mice.

Methods Employed: Total body radiation and I.V. DMBA are used as initiators of neoplasia. Immune manipulations consist of cyclic administration of cortisone acetate as an inhibitor and metanol-soluble fraction of Mycobacterium Butyricum as a stimulator. Age is studied by comparison of 3 month, 9 month, and 15 month old mice at time of initiation of study. Analysis of Bleomycin as a specific local, as well as systemic, initiator of sarcomas and other tumors and relationship to immune suppression.

Major Findings: Delay of onset of lymphomas and acceleration of development and incidence of carcinomas and sarcomas is seen with immune suppression for both radiation and chemical induction. Greater age results in shorter induction periods and changes in tumor incidence patterns. MSFMB has shown no clearcut alteration in control or irradiated animals to date. Bleomycin has produced transplantable undifferentiated sarcomas at the injection site greater than 2 fold increase in other sarcomas. Patterns of development of other tumors indicates a mild immunosuppressive activity of BLM. Tumors peculiar to BLM include early development of sarcomas and very late development of tumors of the adrenal medulla.

Significance to Biomedical Research and the Program of the Institute: Provide knowledge of immune components of the carcinogenic process by radiation and chemicals and the oncologic potential of current chemotherapeutic agents.

Proposed Course: Complete present carcinogenesis studies and analysis of tumor production and patterns. Continue observation of immune stimulation studies and age related immunosuppression for remainder of life span of animals. Prepare results for publication.

COMMITTEE ON SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 00988-11 R

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Study of Chronic Lymphocytic Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Johnson, M.D.	Chief	R	NCI
OTHER:	Staff of the Rad. Onc. Branch		R	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	.5

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the natural history of chronic lymphocytic leukemia, to define those disease parameters which indicate the need for early therapeutic intervention, and to investigate new therapeutic approaches which will improve the quantity and quality of survival.

PHS-6040
(12-75)

Project Description:

Objectives: The study is directed toward understanding the epidemiology, natural history, and management of chronic lymphocytic leukemia.

Methods Employed: One hundred patients with previously untreated chronic lymphocytic leukemia have been accessioned into this study. Information related to carcinogenic exposure, family history, the natural history of disease, immunologic parameters, treatment and response (with primary emphasis on total body irradiation), and survival is being collected and computerized for analysis.

Major Findings: It has been determined for the first time that treatment can alter the natural history of patients with active, progressive chronic lymphocytic leukemia and not only prolong survival but improve the quality of life for approximately one-third of patients.

Significance to Biomedical Research and the Program of the Institute: This study has provided the first documented evidence that treatment of patients with chronic lymphocytic leukemia can quantitatively and qualitatively alter the course of disease.

Proposed Course: Sequential studies are being undertaken to investigate methods of improving upon the treatment program and to determine, if possible, which patients in the indolent phase of their disease warrant early therapeutic intervention.

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Johnson, R. E.: Total body irradiation of chronic lymphocytic leukemia: Relationship between therapeutic response and prognosis. Cancer(In Press).

Johnson, R. E., and Ruhl, U.: Treatment of chronic lymphocytic leukemia with emphasis on total body irradiation. Intern. J. Rad. Oncol.(In Press).

Braylan, R. C., Jaffe, E. S., Burbach, J. W., Frank, M. M., Johnson, R. E., and Berard, C. W.: Similarities of surface characteristics of neoplastic well-differentiated lymphocytes from solid tissues and from peripheral blood. Cancer Res.(In Press).

WITH NATIONAL SCIENCE INFORMATION EXCHANGE SERIAL OR ACCESSION NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 00989-11 R

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Study of Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Johnson, M.D.	Chief	R NCI
OTHER:	Staff of the Rad. Onc. Branch		

COOPERATING UNITS (if any)

Medicine Branch, NCI
Laboratory of Pathology, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	.5

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the natural history of Hodgkin's disease, to assess the reliability of diagnostic methods as they apply to the treatment decisions, to examine the effectiveness of radiotherapy in the management of disease, and to evaluate the long-term survival and the iatrogenic effects of therapy.

Project Description:

Objectives: To understand the natural history of Hodgkin's disease, assess the reliability of diagnostic methods as they apply to the treatment decisions, to examine the effectiveness of radiotherapy in the management of disease, and to evaluate the long-term survival and the iatrogenic effects of therapy.

Methods Employed: A collaborative effort involving a number of cooperating units was undertaken to document the biological characteristics of each patient's disease including the extent of involvement. Subsequently, patients were assigned to various treatment options to test the comparative therapeutic efficacy.

Major Findings: During the past year, there has been no significant change in previously reported survival rates. Careful assessment has been undertaken of the long-term effects of treatment with particular emphasis on the hematologic and immunologic aspects.

Significance to Biomedical Research and the Program of the Institute: This study has demonstrated the merit of extensive prophylactic irradiation in the management of clinical early stage Hodgkin's disease. Information has also been forthcoming as to the relative accuracy of various diagnostic tests and the definition of which situations require unusual pretreatment procedures such as staging laparotomy.

Proposed Course: Continued long-term observation of patients without accession of additional patients to study.

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Gutensohn, N., Li, F., Johnson, R. E., and Cole, P.: Hodgkin's disease. Tonsillectomy and family size. N. Engl. J. Med. 292: 22-25, 1975.

Canellos, G. P., DeVita, V. T., Arseneau, J. C., Whang-Peng, J., and Johnson, R. E.: Second malignancies complicating Hodgkin's disease in remission. Lancet 1: 947-948, 1975.

Kun, L. E., and Johnson, R. E.: Hematologic and Immunologic status in Hodgkin's disease five years after radical radiotherapy. Cancer 36: 1912-1916, 1975.

Johnson, R. E., Glover, M., Johnson, S. K., and Ruhl, U.: Split-course radiotherapy of Hodgkin's disease: Local tumor control and normal tissue reactions. Cancer(In Press).

Kun, L. E., DeVita, V. T., Young, R. C., and Johnson, R. E.: Treatment of Hodgkin's disease using intensive chemotherapy followed by irradiation. Intern. J. Rad. Oncol.(In Press).

ARTICLES PUBLISHED IN A BOOK

Johnson, R. E.: The Role of Selective Staging Laparotomy in Hodgkin's Disease and the Malignant Lymphomas. In Varco and Delaney (Ed.): Controversy in Surgery. Philadelphia, W. B. Saunders (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 00990-11 R

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Study of Non-Hodgkin's Lymphomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Johnson, M.D.	Chief	R NCI
OTHER:	H. D. Brereton, M.D.	Head, Rad. Med. Sec.	R NCI
	Staff of the Rad. Onc. Branch		R NCI

COOPERATING UNITS (if any)

Medicine Branch, NCI
Laboratory of Pathology, NCI

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to increase our understanding of the natural history of the various malignant lymphomas, to define which diagnostic (staging) methods are requisite for making treatment decisions, and to evaluate various therapeutic approaches which might improve the survival and quality of life for patients with these diseases.

PHS-6040

Project Description:

Objectives: To improve our understanding of the natural history of malignant lymphomas, to define which staging methods are required for effective treatment decision-making, and to evaluate new therapeutic approaches with the aim of improving both survival and quality of life.

Methods Employed: An extensive prospective staging evaluation has been performed on 170 consecutive patients with previously untreated patients. Clinical trials using both radiotherapy and chemotherapy are underway to examine the potential for improving the remission rates and survival of lymphoma patients.

Major Findings: The systemic nature of involvement in the overwhelming majority of patients with non-Hodgkin's lymphoma has been established by this collaborative study with the Medicine Branch and Laboratory of Pathology. This information has served to alter the concepts regarding treatment and the more routine use of systemic therapy has resulted. However, while remissions can be achieved in the majority of patients using either radiotherapy or chemotherapy, relapse eventually occurs in most patients and the investigation of alternative therapeutic measures will be required.

Significance to Biomedical Research and the Program of the Institute: This study has contributed to our understanding of non-Hodgkin's lymphoma primarily in terms of understanding the natural history and providing information as to methods of staging patients (i.e., identifying their location in the overall spectrum of disease). The remaining challenge is to develop more effective means of therapy in the future based on the above understanding.

Purposed Course: Continued accession of patients is anticipated for the purpose of examining new treatment approaches.

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Canellos, G. P., DeVita, V. T., Young, R. C., Chabner, B. R., Schein, P. S., and Johnson, R. E.: Therapy of advanced lymphocytic lymphoma: A preliminary report of a randomized trial between combination chemotherapy (CVP) and intensive radiotherapy. Br. J. Cancer 31: 474-480, 1975.

Johnson, R. E.: Total body irradiation (TBI) as primary therapy for advanced lymphosarcoma. Cancer 35: 242-245, 1975.

Brereton, H. D., Anderson, R., Johnson, R. E., and Schein, P.: Hyperphosphatemia and hypocalcemia following chemotherapy of Burkitt's lymphomas. Arch. Intern. Med. 135: 307-311, 1975.

Johnson, R. E.: Management of Generalized Malignant Lymphomata with "systemic" radiotherapy. Br. J. Cancer 31: 450-455, 1975.

Johnson, R. E., DeVita, V. T., Kun, L. E., Chabner, B. R., Chretien, P. B., Berard, C. W., and Johnson, S. K.: Patterns of involvement with malignant lymphomas and implications for treatment decision making. Br. J. Cancer 31: 237-241, 1975.

Chabner, G. A., Johnson, R. E., Chretien, P., Schein, P. S., Young, R. C., Canellos, G. P., Hubbard, S. H., and DeVita, V. T.: Percutaneous liver biopsy, peritoneoscopy, and laparotomy: An assessment of relative merits in the lymphomas. Br. J. Cancer 31: 242-247

ARTICLES PUBLISHED IN A BOOK

Johnson, R. E.: The Role of Selective Staging Laparotomy in Hodgkin's Disease and the Malignant Lymphomas. In Varco and Delaney (Ed.): Controversy in Surgery. Philadelphia, W. B. Saunders(In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 00992-02 R

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Kinetic Studies of DNA Synthesis in Host and Tumor Tissues Following Chemotherapy and Radiation Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. D. Brereton, M.D.	Head, Rad. Med. Sec.	R NCI
OTHER:	T. L. Bryant, B.A.	Biologist	R NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Radiation Oncology Branch

SECTION
Radiation Medicine Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	.25	1

SUMMARY OF WORK (200 words or less - underline keywords)

B_{16} melanoma in BDF_1 mice will be treated with Methyl CCNU and/or radiation therapy. Alterations in DNA synthesis following treatment will be measured to identify a.) the best treatment schedule and combination, and b.) to prevent the rapid development of resistance of this tumor to these treatment modalities.

PHS-6040
(12-75)

Project Description:

Objectives: To evaluate the kinetic changes induced in normal tissues and tumorous tissues following chemotherapeutic intervention and to design optimal drug schedules with this information.

Methods Employed: BDF_1 mice bearing B_{16} melanomas were treated with methyl CCNU. Alterations in DNA synthesis in host tissues and tumor tissues were measured.

Major Findings: An optimal dose schedule for methyl CCNU in this system would have been approximately every six days; however, the rapid resistance is permanent, lasting through many generations. The methods employed may provide a ready means of evaluating drug efficacy in solid tumors in other animal systems.

Significance to Biomedical Research and the Program of the Institute: The proper sequencing of radiation and drugs or successive cycles of chemotherapy is unknown in almost any animal or clinical situation. These studies are designed to explore ways to optimize such drug and drug/radiation treatment schedules.

The major clinical thrust of the Branch is the empiric derivation of optimally tolerated schedules of drugs or drugs and radiation in the treatment of human cancer.

Proposed Course: Continue this work in other solid tumors with other chemotherapeutic agents and radiation therapy.

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Brereton, H. D., Bryant, T. L., Young, R. C.: Inhibition and recovery of DNA synthesis in host tissues and sensitive and resistant B16 melanoma after 1-(2-Chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea, a predictor of therapeutic efficacy. Cancer Res. 35: 2420-2425, 1975.

Young, R. C., Rosenoff, S. A., Myers, C. E., Brereton, H. D., Chabner, B. A.: Alterations in DNA synthesis induced by chemotherapeutic agents in vivo: Potential applications to clinical treatment schedules. M.D. Anderson Monogr: M.D. Anderson Symposium on Fundamental Cancer Research(In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 00994-05 R

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Study of Small Cell Cancer of the Lung

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Johnson, M.D.	Chief	R NCI
OTHER:	H. D. Brereton, M.D.	Head, Rad. Med. Sec.	R NCI
	C. H. Kent, M.D.	Senior Investigator	R NCI

COOPERATING UNITS (if any)

Diagnostic Radiology Dept., CC, NIH
Nuclear Medicine Dept., CC, NIH

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	.5

SUMMARY OF WORK (200 words or less - underline keywords)

This study is concerned with the evaluation of methods for pretreatment assessment of patients, identification of factors influencing response to therapy, and investigation of combined modality treatment using radiotherapy and intensive chemotherapy.

PHS-6040

Project Description:

Objectives: The major objective of the program is to improve the survival time for patients with small cell carcinoma of the lung and hopefully to develop treatment programs which may offer some prospect of cure.

Methods Employed: Thirty-one patients with previously untreated disease have been accessioned into the study in the past 18 months. Pretreatment evaluation has been confined primarily to the assessment of those factors which may complicate management and/or influence tolerance to therapy. The therapeutic approach utilizes concurrent radiotherapy to regional disease, combination chemotherapy, and CNS prophylaxis.

Major Findings: The basis for the treatment program as employed is assumption that all patients have widely disseminated disease at the time of diagnosis and approximately one-half of the patients entered into study have had documented extrathoracic spread. Initial response to treatment has been encouraging with a 90% complete remission rate following the 12 week remission induction phase. No maintenance treatment has been given and approximately one-half of patients have remained in unmaintained remission following cessation of therapy with maximum observation periods of one year to date.

Significance to Biomedical Research and the Program of the Institute: There has been no major improvement in the treatment of lung cancer until the present time and this program will constitute a significant advance if patients continue to have durable, unmaintained remissions for a significant period of time.

Proposed Course: Accession of patients is continuing with the goal of improving the disease-free rate and developing techniques for combining chemotherapy and radiotherapy without incurring serious toxicity as experienced by a significant fraction of patients treated during the early phase of the study.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 00995-01 R

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Radiation Therapy Planning Using Computerized Tomography Interfaced with Computerized Dosimetry Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. R. Kirkland, Ph.D.	Physicist	R	NCI
OTHER:	R. E. Johnson, M.D.	Chief	R	NCI
	D. Syed, M.S.	Head, Systems Design Sec.	CR	NIH
	W. L. Risso, M.E.	Elec. Engineer	CR	NIH

COOPERATING UNITS (if any)
Computer Systems Laboratory, CR, NIH

LAB/BRANCH
Radiation Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	3	0

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been initiated during the past year in an effort to exploit the potential of computerized tomography for radiation therapy. An EMI whole-body scanner is being installed at present and procurements are underway for ancillary computer equipment to permit interfacing of the CT scanner to a computerized dosimetry treatment planning system. The obvious implications will be not only to obtain improved imaging resolution of tumors but to correct for tissue inhomogenities in the planning of radiation therapy. It also appears that "state of the art" technology will afford the potential for three-dimensional treatment planning as a necessary prerequisite for computer-assisted treatment delivery.

PHS-6040

Project Description:

Objectives: To utilize the imaging from computerized tomography for improved accuracy in radiation treatment planning and to employ data on tissue densities inherent in the scanning process to correct for tissue inhomogeneties in dosimetry planning.

Methods Employed: A closely integrated effort is being undertaken by the staff of the Division of Computer Research and Technology and the Radiation Oncology Branch to implement this study. The whole-body CT scanner is presently being installed and most of the major ancillary equipment has been requisitioned. Evaluation of existing dosimetry programs is already underway together with examination of many interrelated aspects such as display systems, problems with interfacing the scanner with computer dosimetry systems, etc.

Major Findings: This project has only been initiated during the past six months and implementation has yet to begin awaiting arrival and installing of the above units.

Significance to Biomedical Research and the Program of the Institute: A successful outcome to this program will improve the sophistication of radiation therapy throughout the world by a significant order of magnitude.

Proposed Course: Expedient prosecution.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06502-11 R

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Adjuvant Chemotherapy and Immunotherapy of Ewing's Sarcoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. C. Pomeroy, M.D.	Head, Rad. Ther. Sec.	R NCI
OTHER:	R. E. Johnson, M.D.	Chief, Rad. Onc. Branch	R NCI
	Staff of Rad. Onc. Branch		R NCI

COOPERATING UNITS (if any)

Laboratory of Immunodiagnosis, DCBD, NCI; Laboratory of Pathology, DCBD, NCI; Laboratory of Biomedical Sciences, IRP, NICHD; Litton Bionetics, Inc.; Department of Medicine, George Washington University; AFRRI.

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to evaluate the combined modality treatment of Ewing's sarcoma as a model system of experimental therapeutics and to further evaluate the immunologic factors involved in the disease and its treatment, and determine the value of adjunctive immunotherapy to the successful combined radiation chemotherapy program.

PHS-6040

Project Description:

Objectives: To determine the value of adjunctive chemotherapy and immunotherapy in the management of Ewing's sarcoma.

Methods Employed: Multiple drug chemotherapy, intrathecal chemotherapy and whole brain irradiation, immunotherapy with BCG and irradiated allogeneic tumor cells are combined with local radiation to the primary and metastatic sites in patients referred with a diagnosis of Ewing's sarcoma. Immune competence is monitored by skin testing and in vitro studies.

Major Findings: Adjuvant therapy has now produced a greater than 70% projected disease free survival for those patients appearing without clinically detectable metastasis. Immune competence as monitored by tumor antigen skin testing, immunization to KLH, LMI with tumor antigens, and T-cell rosetting has related well to prognosis. Preliminary indications in metastatic and relapsed patients indicate improved tumor control, survival and tolerance of chemotherapy with BCG immune stimulus. Glycoprotein profiles have not proved to be sufficiently useful in prognosis and monitoring and will be discontinued. LDH and isoenzymes have continued to hold high initial prognostic value and have indicated probable relapse in a few patients months before detectable by any other means.

Significance to Biomedical Research and the Program of the Institute: Provides increased evidence of the value of adjunctive chemotherapy and immunotherapy in maintaining disease free state in this model system for combined modality treatment.

Proposed Course: Continue patient acquisition for further evaluation of treatment methods and to determine significance of effects of immunotherapy. To pursue preliminary leads as to the role of hormones in age-specific incidence and relapse. To refine immunologic methodology including development of serologic tests with present relatively specific antigens from recently established tumor cell lines. It is anticipated these cell lines will provide an unlimited source of antigenic material for diagnostic and therapeutic use.

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Brereton, H.D., Simon, R., and Pomeroy, T.C.: Pretreatment serum lactate dehydrogenase predicting metastatic spread in Ewing's sarcoma. Ann. Intern. Med. 83 (3):352-345, September, 1975.

Mittel., H.S., and Pomeroy, .: Iatrogenic fractures of biopsy sites in long bon . of Bone and Joint Surg. (In press).

Johnson, R.E., and Pomeroy, T.C.: Evaluation of therapeutic results in Ewing's sarcoma. Am. J. Roentgenol., Radium Ther., and Nucl. Med. 123 (3):583-587, March, 1975.

Pomeroy, T.C., and Johnson, R.E.: Prognostic factors for survival in Ewing's sarcoma. Am. J. Roentgenol., Radium Ther., and Nucl. Med. 123 (3):598-606, March, 1975.

Zimbler, H., Robertson, G.L., Bartter, F.C., Delea, C.S., and Pomeroy, T.C.: Ewing's sarcoma as a cause of SIADH. J. Clin. Endocrinol. Metab. 41 (2):390-391, August, 1975.

ARTICLES IN A BOOK:

Johnson, R.E., and Pomeroy, T.C.: Integrated therapy for Ewing's sarcoma. In Ariel, I.M. (Ed.): Prog. Clin. Cancer. New York, Grune & Stratton, 1975, vol. VI, pp. 95-97.

Pomeroy, T.C., and Johnson, R.E.: Integrated therapy of Ewing's sarcoma. In Vaeth, J.M. (Ed.): Proceedings of the Tenth Annual San Francisco Cancer Symposium. (September 27-28, 1974). Frontiers of Radiation Therapy and Oncology. New York, S. Karger, 1975, vol. 10, pp. 152-166.

ANNUAL REPORT SUMMARY

SURGERY BRANCH

NATIONAL CANCER INSTITUTE

July 1, 1975 through June 30, 1976

This has been a year of continuing transition for the Surgery Branch. On July 1, 1974 Dr. Steven A. Rosenberg became Chief of the Surgery Branch, replacing Dr. Alfred S. Ketcham, who had held that position for the past 17 years. With Dr. Rosenberg came a change in philosophy and emphasis within the Surgery Branch. There have been significant changes in senior staff personnel, organization of the clinical and teaching services and changes in research directions.

In accordance with Dr. Rosenberg's emphasis on combined modality treatment and the strong involvement of surgical oncologists in the postoperative adjunctive modalities of immunotherapy and chemotherapy, a shift in emphasis towards these directions is in progress. The future development of the Surgery Branch will be guided by the principle that the future of surgical oncology lies not in the direction of bigger and wider cancer resections but rather with a strong commitment to and involvement in adjunctive treatment.

The realization that two-thirds of patients already have disseminated disease at the time they first consult a surgeon requires that the surgeon gain expertise in the control of disseminated disease as well as the local control of cancer.

Towards this end, the clinical and laboratory efforts of the Surgery Branch will be devoted to the development of innovative immunologic and other approaches to the treatment of malignant disease to be used as surgical adjuvants. The Surgery Branch will also place emphasis on its involvement in the administration of these adjuncts in many of its protocols.

In keeping with this philosophy the Surgery Branch this year submitted six protocols to the NIH Clinical Research Committee involving the combined modality treatment of cancer. These protocols have involved the treatment of primary malignant melanoma, the treatment of soft tissue sarcomas of the extremities, the treatment of soft tissue sarcomas of the head and neck and trunk, the treatment of osteogenic sarcoma, the treatment of patients with thoracic malignancies receiving thymosin, and the treatment of patients with colorectal cancer. Several of these protocols involve the joint participation of the Surgery, Immunology, Radiation Oncology, and Medical Oncology Branches. These protocols are evaluating a variety of immunologic, as well as chemotherapeutic adjuvants to major surgical treatment. The Surgery Branch is involved in an extensive review of its clinical activities in an attempt to develop innovative combined modality treatment protocols for a variety of diseases.

During the past year Dr. Murray Brennan joined the Surgery Branch as Head of the newly-created Surgical Metabolism Section. Dr. Brennan is nationally

known in the fireld of surgical metabolism and hyperalimentation and has developed clinical and laboratory programs in this area oriented toward elucidating nutritional factors important in tumor-host reltionships and their possible role in the management of patients with cancer. Dr. Brennan has organized a clinical service in nutrition and intravenous hyperalimentation that has greatly improved clinical care in this area at the National Institutes of Health.

Much of the research in the Surgery Branch has also undergone a change in direction. The arrival of Dr. Rosenberg, Dr. Alan Baker and Dr. Murray Brennan, all experienced clinicians as well as research scientists, has led to the development of many new studies in the basic biology of the tumor-host relationship.

Surgical procedures and consultations performed by the Surgery Branch from April 1, 1975 to March 31, 1976 are presented in Tables I through IV.

TABLE I

Surgical Procedures in the Surgery Branch*

April 1, 1975 - March 31, 1976

General	
Exploratory laparotomy	33
Splenectomy	17
Colectomy	18
Abdominoperineal resection	4
Gastrectomy	1
Pancreas (resection or bypass)	4
Incisional hernia	2
Cholecystectomy	7
Appendectomy	1
Major soft tissue resection	20
Hemorrhoidectomy	1
Herniorrhaphy	5
Miscellaneous	30
Melanoma:	
Wide excision	26
LND	39
WE + LND	51
Cryosurgery	1
Head and Neck	
Radical neck dissection ± other resection	10
Other major cases	12
Parotid	1
Flap revisions	4
Tracheotomy	6
G-U	
Renal	15
Reconstruction ileal conduit	1
Prostatectomy	5
Cystectomy	1
Other - general	19
Cystoscopy	50
Orchiectomy	4
Breast	
Biopsy	58
Mastectomy	13
Oophorectomy	8
Subcutaneous mastectomy	10

TABLE I (cont'd.)

Amputations	
Above-knee	26
Hip disarticulation	6
Hemipelvectomy	5
Forequarter	2
Stump revision	1
Gynecologic	
Exenterations	4
Simple hysterectomy	5
D&C	7
Tubal ligation	3
Endocrine	
Parathyroid	22
Thyroid	20
Adrenal tumors	7
Endoscopy	
Colonoscopy	8
Fiberoptic laryngoscopy	1
Looposcopy	4
Esophagoscopy	2
Minor	
Biopsies	199
I&D abscess	13
Minor Plastic Repairs	14

*In compiling this list the major aspect of each procedure was used. Patients with multiple simultaneous procedures appear on the list only once.

TABLE II
Consultants - Surgery
April 1, 1975 - March 31, 1976

Plastic	5
Thoracic	
Pericardectomy	5
Bronchoscopy	8
Thoracotomy - nodules	30
Thoracotomy - infiltrate	22
Thoracotomy - other	1
Mediastinoscopy	6
Pneumonectomy	5
Gynecologic	
D&C	1
Miscellaneous	2
ENT	19
Miscellaneous	
A/U shunt, epigastric hernia, esophagostomy	6

TABLE III
ENT Consultations
April 1, 1975 - March 31, 1976

	General	Audiograms	Biopsies
April	47	34	4
May	35	22	6
June	29	24	8
July	46	31	1
August	45	30	0*
September	29	26	0
October	35	24	0
November	33	29	0
December	46	27	0
January	35	29	0
February	26	16	0
March	59	23	0
Totals	465	315	19

*No biopsies since Dr. Henkin left the Clinical Center.

TABLE IV

Individual Written Intramural Consultations Received by Surgery Branch, NCI
Listed by Institutes*
April 1, 1975 - March 31, 1976

NCI	132
NIAMDD	71
NHLI	36
NIAID	36
NIMH	36
NINCDS	33
NIDR	12
NICHHD	8
NEI	1
TOTAL	365

*This tabulation includes only formal written consultations submitted to the Surgery Branch office. Many additional consultations are received by Surgery Branch physicians directly by telephone or personal contact. These are not included in this tabulation.

TABLE V

Annual Report of NIH Surgical Suites

Listing the Number of Major and Minor Procedures

Carried Out by Each of the Operative Units at NIH

April 1, 1975 - March 31, 1976

Patient		NCI	NHLI	NINCDS Neuro.	NINCDS Muscle	NIAID	NIAMDD	NEI	NIMH	NICHHD	NIDR	Consult-tants	Total Pts Operated	# Pts
NCI	Major	416	41	10	1	--	--	--	--	--	--	9	477	823
	Minor	333	6	1	3	--	--	--	--	--	--	3	346	
NHLI	Major	29	259	--	--	--	--	--	--	--	3	-	291	324
	Minor	10	19	--	2	--	--	--	--	--	1	1	33	
NINCDS	Major	9	17	54	--	--	--	--	--	-	--	3	85	309
	Minor	13	1	2	208	--	--	--	--	--	--	--	224	
NIAMDD	Major	54	4	--	--	--	--	--	--	--	--	7	65	97
	Minor	23	--	--	3	--	--	--	--	--	--	6	32	
NIAID	Major	18	3	1	--	--	--	--	--	--	--	3	25	62
	Minor	22	--	1	4	--	--	--	--	--	--	10	37	
NIDR	Major	--	--	--	--	--	--	--	--	--	1	--	1	4
	Minor	2	--	--	1	--	--	--	--	--	--	-	3	
NIMH	Major	5	--	--	--	--	--	--	--	--	--		5	8
	Minor	2	--	--	1	--	--	--	--	--	--	-	3	
NICHHD	Major	19	--	1	--	--	--	--	--	4	--	--	24	31
	Minor	5	--	--	2	--	--	--	--	1	--	--	7	
NEI	Major	1	--	--	--	--	--	21	--	--	--	1	23	35
	Minor	--	--	--	2	--	--	10	--	--	--	--	12	
MAJOR TOTAL		551	324	66	3	0	0	21	0	4	4	23	996	
MINOR TOTAL		410	26	4	226	0	0	10	0	0	1	20	697	
GRAMD TOTAL		961	350	70	229	0	0	31	0	4	5	43	1693*	
												10A Cardiac Cath. Lab	316	
												7th Fl. Cardiac Cath. Lab	291	
													2300	

*Of these 1693 surgical procedures, 117 patients were on OPD status.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03800-06 SURG

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Surgical Consultations and Collaborative Research Involving Surgical Services at the National Institutes of Health

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG NCI
	and entire staff of	Surgery Branch, NCI	SURG NCI
OTHER:	G. D. Aurbach	Chief, Metabolic Dis. Br.	MD NIAMDD
	F. C. Bartter	Clinical Director	IR OD NHLI
	J. L. Doppman	Chief, Diag. Radiol. Dept.	DR CC
	R. E. Johnson	Chief, Rad. Oncol. Br.	RO NCI
	J. Robbins	Chief, Clin. Endocrin. Br.	CE NIAMDD
	L. B. Thomas	Chief, Lab. of Pathology	LP NCI
	R. C. Young	Chief, Medicine Branch	M NCI

COOPERATING UNITS (if any)

Metabolic Diseases Branch, NIAMDD; Division of Intramural Research, OD, NHLI; Diagnostic Radiology Dept., CC; Radiation Oncology Branch, NCI; Clinical Endocrinology Branch, NIAMDD; Laboratory of Pathology, NCI; Medicine Branch, NCI

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

The Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients for elective consultations as well as all emergency general surgical problems. Many collaborations on clinical studies have resulted from these consultative efforts, including studies of hyperparathyroidism, thyroid disease, as well as study of a variety of other endocrine problems.

Introduction

Investigators in the Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients in primarily two capacities. Firstly, we see patients in consultation for all general surgical and specialty surgical problems except for the specialties of cardiac and orthopedic surgery. The Surgery Branch answers all emergency as well as elective surgical consultations and provides 24 hour coverage for surgical emergencies that may arise in the Clinical Center Hospital.

Secondly, the Surgery Branch collaborates in the procurement of tissues for studies required by other investigative units. The degree of involvement of the Surgery Branch in the planning and execution of these studies is variable. The Surgery Branch often plays an instrumental role in the design of these studies while in other collaborations, the Surgical Service merely provides tissues.

Approximately 40% of the clinical surgical effort of the Surgery Branch is devoted to these consultative and collaborative studies.

There is a particular emphasis in our collaborative studies on endocrine diseases. During the past 12 months the Surgery Branch performed operations on 22 patients with parathyroid disease, 20 patients with thyroid disease, and seven patients with adrenal tumors. We performed a total of 199 diagnostic biopsies, most of which were in our capacity as a consultant for other services. A complete listing of our surgical procedures is presented in Table I. Surgery performed by surgical consultants operating within the Surgery Branch is listed in Table II.

The Surgery Branch is heavily involved in providing outpatient ENT consultations through its staff of consulting otolaryngologists. The extent of this work is presented in Table III.

A list of consultations received from other NCI Branches as well as other NIH Institutes is shown in Table IV.

The operative case load in the NIH surgical suites by referring Institute is shown in Table V.

Project Description:

Part I. Hyperparathyroidism

Part II. Thyroid Studies

Part III. Reduction Mammoplasty

Part IV. Evaluation of the Countercurrent Exchange of Testosterone in the Spermatic Artery and Vein of Men with Infertility

Part V. Nutritional Support

Part VI. Surgery for Pulmonary Malignancies

Part VII. Innovative Surgical Techniques for Correction of Renovascular Hypertension

Project Description:

Part I. Hyperparathyroidism

Objectives:

The Surgery Branch of the National Cancer Institute is actively involved in a major clinical commitment with the Section of Mineral Metabolism of the Metabolic Diseases Branch of the National Institute of Arthritis, Metabolism and Digestive Diseases and the Diagnostic Radiology Branch of the Clinical Center, and to a lesser extent the Clinical Endocrinology Branch of the National Heart and Lung Institute, of the study and treatment of hyperparathyroidism. The Surgery Branch provides the end therapy of an extensive investigative protocol involving clinical, laboratory, and specialized radiographic techniques designed to delineate the need for surgery and to attempt to localize the site of abnormal parathyroid glands.

A major new area being explored is the use of parathyroid transplantation to treat patients who would have normally been rendered aparathyroid by their operation. This becomes particularly pertinent as many of the cases that we operate on have been previously operated on elsewhere. The surgery provides a particular technical challenge and requires a major committal of time.

Methods Employed:

Standard protocols for investigation are currently in action. Current emphasis on diagnosis has been the use of thyroid arteriography and parathyroid venous drainage sampling.

Major Findings:

From January 1 until August 19, 1975, seven cases were operated on. From August 1 until April 1, 15 cases have been operated on. Of these, 11 have had previous operations. Of those 11, six had had two previous neck explorations and of those six, four had also had a previous mediastinal exploration. Fourteen of 15 have had their abnormal gland identified and removed (Table 6). One patient who had an indwelling tracheostome had a wound infection and a second patient who previously had a left recurrent nerve divided, had an elective tracheostomy placed as the right nerve had not been clearly identified. In this same patient the carotid artery was injured during surgery and required carotid end-to-end reanastomosis. This one patient was the one in which the abnormal gland was not found (Table 7). Three patients had been rendered aparathyroid and received forearm parathyroid

Table 6

SURGERY FOR HYPERPARATHYROIDISM

August 1, 1975 to April 1, 1976

Number of Cases Operated	15
Previous Surgery: 21 procedures on 11 patients	
One previous neck exploration	5
Two previous neck explorations	6*
Results	
Successful surgery	14
Aparathyroid	3**
Hyperparathyroid	1
Euparathyroid	10***

*4 also had a prior mediastinal exploration.
**All autotransplanted.
***1 probably aparathyroid.

Table 7

COMPLICATIONS OF SURGERY FOR HYPERPARATHYROIDISM

August 1, 1975 to April 1, 1976

Number of Cases	15
Wound infection	1*
Carotid artery injury	1**
Elective tracheostomy	1**
Remain hyperparathyroid	1**
Recurrent nerve paralysis	1

*Chronic tracheostome.
**Same patient.

transplants. To date evaluation of these is incomplete but they appear to have functioning grafts. A further patient is probably aparathyroid but currently maintained on vitamin D and calcium until he can be further evaluated. Fourteen of 15 had preoperative venous catheterization. This proved helpful in 12 of 14. Arteriography was performed in 11 cases. This was helpful in localization in nine of the 11.

Significance to Cancer Research and the Program of the Institute:

The surgery of this disease is an essential end point in the therapy and management of patients with hyperparathyroidism. By early diagnosis and successful surgery an important step can be taken towards the prevention of the complications of prolonged hypercalcemia. These primarily manifest in our experience with end-stage renal disease. The Institute provides a national service in providing a referral center for those patients previously operated on unsuccessfully elsewhere. By the use of the extensive preoperative evaluation and localization techniques, successful reoperation should be obtainable.

Proposed Course:

This project will continue with the further emphasis by other branches on diagnosis and localization. The Surgery Branch will be actively involved in the development of the technique of cryopreservation, in determining the need for autotransplantation, and the subsequent evaluation of the course of patients and their autografts.

Project Description:

Part II: Thyroid Studies

Objectives:

There are three primary areas in which the Surgery Branch is involved in the treatment of thyroid disease. (1) The program by the Clinical Endocrinology Branch of the NIAMDD evaluating cold thyroid nodules. (2) The general consultative service for thyroid disease, usually cancer, discovered in other Institutes. (3) The interest of the Surgery Branch in primary and metastatic medullary carcinoma of the thyroid gland.

Methods Employed:

(1) The Clinical Endocrinology Branch of the NIAMDD has an ongoing protocol to evaluate cold thyroid nodules. This protocol involves initial diagnostic scans, aspiration and cytology of the nodule, treatment with a short one week course of prednisone followed by a two to three month course of suppressive doses of thyroxine. The failure of the nodules to resolve or completely disappear by six months results in surgical referral. Twenty-five of these patients have come to surgery over the last two years for definitive diagnosis and treatment of the thyroid nodule.

(2) This involves the remaining number of thyroid cases.

(3) The Surgery Branch has recently been involved in two aspects of medullary carcinoma of the thyroid gland.

a. In an effort to delineate those patients who have residual metastatic disease, basal thyrocalcitonin levels have been established in those patients whose levels have been above normal and an attempt has been made to identify their residual disease by central vein catheterization with the support and expert assistance of Dr. John Doppman (Clinical Radiology).

b. In addition, the Branch has recently been introduced to a propositus of a new family of familial medullary carcinoma of the thyroid. It is the present intention of the Branch to continue to screen family members of this rather large family for as yet undiagnosed disease. This may well involve the committal of a considerable amount of time and effort to the setting up of a thyrocalcitonin assay which is not currently available within the National Institutes of Health.

Major Findings:

(1) In the past two and a half years 63 patients with cold nodules have been entered into the study. Twenty-five patients have been operated on, and of these 25, 9 have had cancer. The data is currently being evaluated by Dr. Gershongon and Dr. Robbins of NIAMDD.

(2) In the last year the Surgery Branch has operated on 20 thyroid cases (includes those cases operated on in (1) above).

(3) a. Present indications suggest that the central venous sampling technique may well be of value in the diagnosis of and localization of recurrent disease.

b. It remains to be seen whether the thyrocalcitonin assay will be set up within the Branch. It is an area that deserves further study as this is a cancer which is currently unresponsive to radiotherapy and chemotherapy but for which surgery can provide a cure and, more importantly, it is a carcinoma that has a biological marker (thyrocalcitonin) which allows accurate evaluation for recurrent disease.

Significance to Cancer Research and the Program of the Institute:

The thyroid studies as currently performed are a major service function of the Branch. They provide important research confirmation for the cold nodule thyroid study and are beginning to provide important definitive information in the medullary carcinoma of the thyroid group.

Proposed Course:

The first two groups will continue to be cared for by the Surgery Branch as part of ongoing protocols of other branches. The third area involves a considerable amount of expansion and effort if it is going to provide relevant data; at the present time it provides important patient management

information but does not provide a great deal of new and active research material.

Project Description:

Part III. Reduction Mammoplasty

Objectives:

In collaboration with the National Institute of Child Health and Human Development, the Surgery Branch, National Cancer Institute, is undertaking a collaborative study on male patients with gynecomastia. This program is intended to offer patients who are undergoing workup in the National Institute of Child Health and Human Development for their gynecomastia an opportunity to have reduction mammoplasty undertaken as a part of their treatment and rehabilitation. The purpose of this procedure is to reduce the size of the breast to a normal male contour and to obtain specimens of breast tissue for routine pathology examination and for analysis for estrogen receptors.

Methods Employed:

Reduction mammoplasty is undertaken under general anesthesia in the operating suite of the Surgery Branch, National Cancer Institute. Two techniques have been employed for removal of fat and breast tissue. An inframammary incision which affords wide exposure and direct visualization of tissue to be removed was used initially. This resulted in greater postoperative scarring than a recently adopted procedure. The second technique involves use of a circumareolar incision extending approximately 180° around the areola. This affords limited visualization of tissue removed but does have the advantage of producing a less noticeable scar.

Major Findings:

Results of operations to date indicate that both methods are satisfactory although the circumareolar incision does afford less noticeable scarring. There has been a tendency to over-correct some patients while on the other hand, some have been under-corrected. Overall the results have been satisfactory and continued effort will be made to improve the surgical techniques.

All of the procedures have afforded adequate samples of breast material for study by the National Institute of Child Health and Human Development.

Significance to Cancer Research and the Program of the Institute:

Some of the patients operated have Klinefelter's syndrome and have been of special interest in this study since these patients are thought to have a higher incidence of male breast cancer. Information gathered through this program may be of value in giving added insight into the occurrence of male breast cancer.

Proposed Course:

This study will be continued to afford continued consultative support for the program of the National Institute of Child Health and Human Development. Surgical techniques will be improved with further experience.

Project Description:

Part IV. Evaluation of the Countercurrent Exchange of Testosterone in the Spermatic Artery and Vein of Men with Infertility

Objectives:

Recent studies in the rat and monkey have demonstrated a higher concentration of testosterone in the spermatic artery than in the peripheral circulation. The data in primates from our laboratory has suggested that this concentration gradient results from a countercurrent transfer of testosterone to the artery from surrounding testicular veins (Table 8). These findings are provocative in view of the known requirement of high intratesticular testosterone levels for maintenance of spermatogenesis. The present study is designed to assess whether levels of testosterone differ between femoral artery and testicular artery in man, and additionally to determine if the testosterone concentration increases along the course of the artery as it approaches the testis. The study will assess the countercurrent exchange mechanism in normal men, men with infertility associated with varicocele, and men with idiopathic infertility.

Methods Employed:

Two groups of patients are being studied. A group of apparently normal males, ages 18-40, who are to undergo surgical procedures which require exposure of the spermatic cord are asked to volunteer for the study. A complete evaluation of reproductive function is performed. At the operation, blood is drawn from the femoral artery and vein and from the distal and proximal spermatic artery and vein. A second group of infertile men, participants in an ongoing comprehensive study of male infertility, who require an operative procedure for evaluation of infertility (such as testicular biopsy), are likewise asked to volunteer for the intra-operative blood sampling. This second group is to include patients with varicoceles and with idiopathic infertility.

The blood samples are assayed for testosterone, cortisol, FSH, LH, total protein, and urea nitrogen.

Major Findings:

At this time, preliminary data are available from three patients: one undergoing hydrocelectomy, one orchiectomy for breast cancer, and one varicocelectomy. In each case, the distal spermatic artery concentration of testosterone was higher than the proximal arterial concentration (606 vs. 505, 342 vs. 232, and 197 vs. 153 ng/100 ml). Furthermore, in two of the

Table 8

AVERAGE CHANGE IN [T] NG/100 ML IN SPERMATIC ARTERY

	Peripheral	Proximal	Middle	Distal	N	$\alpha\bar{X}$	SEM	T	p	%Δ[T]
$\bar{X}$ SEM	104 ±13 →		102 ±12		27	−2	2	−0.29	NS	0
	104 ±13 →			120 ±17	29	+16	8	2.30	=.03	+16%
		100 ±12 →	102 ±12		32	+2	2	1.97	NS	0
		100 ±12 →		120 ±17	30	+20	9	2.775	<.01	+20%
			102 ±12 →	120 ±17	28	+18	7	2.408	=.03	+18%

three cases, the peripheral arterial testosterone concentration was lower than that measured in the proximal artery. No consistent differences in the levels of cortisol, LH, FSH, BUN, or total protein have been found. These preliminary results support the hypothesis that a countercurrent exchange mechanism for testosterone in the spermatic cord of man results in an increased arterial testosterone concentration in the testicular inflow.

Significance to Cancer Research and the Program of the Institute:

This study of testicular physiology in normal men and patients with infertility was designed in an attempt to produce new insights into the pathophysiology of infertility. The subjects of the study are men whose operative care is normally provided by the Surgery Branch as part of its commitment to make consultative service available to the Reproductive Research Branch of the NICHD. The development of this collaborative study is an effort to make these services more productive, and the preliminary results have already demonstrated the existence in man of an exchange mechanism for testosterone in the spermatic cord.

Proposed Course:

The study will be continued to completion as outlined, to determine whether alterations in the spermatic countercurrent exchange of testosterone play a role in infertility secondary to varicoceles. The goal is to have at least ten normal controls, ten patients with a varicocele, and ten patients with idiopathic infertility.

Project Description:

Part V. Nutritional Support

Objectives:

The Surgery Branch has provided a major service commitment in a total parenteral nutrition or intravenous feeding team. This consists of a senior investigator, two clinical associates, a TPN nurse, and the required laboratory and data analysis support. The objective was to fulfill a badly needed service within the National Institutes of Health and to try to develop some meaningful methods of assessing the adequacy of parenteral nutrition. In addition, we expect to develop specific randomized protocols to examine the effectiveness of adjunctive nutritional support to the primary modalities of surgery, chemotherapy, or radiotherapy in the treatment of cancer.

Methods Employed:

The service is set up as a consultant service as with any other consultative service with the Surgery Branch. Patients are seen on referral basis and the decision is made as to whether they will be helped by nutritional support. At the present time, the service has not been aggressive about obtaining patients until firmly established. There seems little doubt that many

patients within the National Institutes of Health remain chronically malnourished and could be helped by the service, but it is not the intention to be more aggressive at the present time. An extensive standardized protocol of line insertion, appropriate nutritive solutions, monitoring procedures, dressing changes, the assessment and treatment of complications has been firmly established.

Major Findings:

From August 1 until April 1, a total of 13 patients have been treated for a range of two-104 days. This has involved the insertion of 29 catheters with a total patient day treatment of 466 days. Ten of these 13 patients have not been patients of the surgical service of the NCI but patients in other parts of the National Institutes of Health (see Table 9). All but one of these patients had at some stage in their background proven cancer. The primary problem remains the incidence of sepsis with five of 29 catheters being incriminated with organisms found both in the central line and in the blood stream.

Significance to Cancer Research and the Program of the Institute:

This is an extensive commitment of the Surgery Branch to the better management of patients. It provides an ongoing commitment to a form of adjunctive therapy that has been proven in areas other than in cancer but is still being evaluated in that area.

Proposed Course:

The Surgery Branch will continue to provide a service to those other sections of the National Institutes of Health prepared to cooperate in a way that would insure the maximum safety of the patient.

Project Description:

Part VI. Surgery for Pulmonary Infiltrates

Objectives:

To determine the usefulness of open lung biopsy in the management of acute, diffuse pulmonary infiltrates occurring in immunocompromised patients.

Methods Employed:

A small anterior thoracotomy is performed and the entire accessible thoracic cavity inspected and palpated. The area for biopsy is selected and a portion of lung is removed using an automatic stapling device. Sixteen open lung biopsies were performed between April 1974 and April 1975 for acute, diffuse pulmonary infiltrates.

Table 9

TOTAL PARENTERAL NUTRITION

August 1, 1975 to April 1, 1976

Number of patients*	13
Number of courses**	18
Total days of TPN	466
Mean days/course	25,89
Range	2→104
Number of catheters inserted	29
Number of catheters used for TPN	26
Mean days TPN/catheter	18.4
Range	2→71

*10 of 13 were not patients of Surgery Branch, NCI.
**3 patients had 8 courses (interval > 10 days off TPN.

Major Findings:

Open lung biopsy can be performed in this group of critically ill patients with postoperative pneumothorax in 7%, serious complications in less than 5%, and no operative mortalities. A specific diagnosis can be made in 71% of patients and appropriate treatment instituted; in the remaining patients therapy with potentially harmful drugs can be avoided.

Significance to Cancer Research and the Program of the Institute:

There is a large group of immunocompromised patients who have received anti-neoplastic drugs, corticosteroids, or who have a primary immunodeficiency disorder. In the past the appearance of diffuse pulmonary infiltrates in these patients usually meant progressive and ultimately fatal pulmonary insufficiency due to unreliable diagnostic techniques and the resulting nonspecific therapy. Open lung biopsy has improved clinical management and treatment of these diseases by rapidly providing suitable tissue for diagnosis thus allowing the institution of specific therapy.

Proposed Course:

The project will continue with further evaluation of diagnostic techniques such as bronchoscopy, transbronchoscopic biopsy, and needle aspiration. The optimal agents for treatment of pneumocystis pneumonia are being evaluated.

Project Description:

Part VII. Innovative Surgical Techniques for Correction of Renovascular Hypertension

Objectives:

Renovascular hypertension resulting from correctable renal arterial lesions are usually treated by an aortorenal bypass or a patch graft of the renal artery. However, conditions such as progressive aneurysms of the renal artery with hypertension, or fibromuscular hyperplasia involving the main renal artery and its branches, present difficult surgical problems. Dissecting aneuysms, especially in the right renal artery, are not readily accessible for *in situ* repair due to the location posterior to the renal vein and the inferior vena cava. Furthermore, the use of the saphenous vein as a graft has resulted in frequent failures due to late aneurysmal dilatation of the graft. We have been attempting to develop techniques (a) to facilitate the delicate and extensive operative repair of the renal artery and its branches by extracorporeal bench surgery, (b) to improve the quality of aortorenal bypass grafts by utilizing autologous hypogastric artery as the graft, and (c) to limit surgery for localized renal lesions to segmental nephrectomy.

Methods Employed:

(a) Extracorporeal repair of renal arterial aneurysm or extensive renal arterial lesions is performed by dividing the renal vessels and bringing the kidney onto the abdominal wall, leaving the ureter intact. After extensive dissection and repair of the renal artery or its branches the kidney is transplanted into the iliac fossa. Renal injury resulting from prolonged ischemia and microemboli of renal arteriolar branches is prevented by perfusion of the kidney and judicious use of systemic heparin.

(b) For the aortorenal bypass, the autologous hypogastric artery, delineated by preoperative angiography, is removed and anastomosed end to side to the aorta and the renal artery, bypassing the stenotic segment of the renal artery.

(c) Selective catheterization of the renal vein branches is performed preoperatively to collect blood for assay of plasma renin activity when a segmental renal lesion is thought to be the source of renin-mediated hypertension. If a significant increase in renin activity is demonstrated in the segment containing the lesion, a partial or segmental nephrectomy can be performed. This operation requires meticulous dissection of the renal parenchyma and complete hemostasis. The calyces and renal capsule are repaired by conventional techniques.

Major Findings:

(a) One patient undergoing extracorporeal bench surgery has remained normotensive without any medication, now seven months after surgery.

(b) The short-term results of the use of autologous hypogastric artery as a vascular graft have been encouraging in two patients. However, nephrectomy was necessary in one patient because of progression of the original disease, fibromuscular dysplasia, into the renal arteriolar tree.

(c) The results of segmental nephrectomies have been rewarding. A follow-up of 1-3 years in four patients in this series revealed all to be normotensive without medication.

Significance to Cancer Research and the Program of the Institute:

These innovative surgical techniques for correction of renal vascular hypertension are essential to the program of the Heart and Lung Institute. The identification of segmental renal lesions responsible for hypertension by selective sampling for renin activity in renal vein branches has made a significant improvement in the diagnosis and therapy of renal hypertension.

Proposed Course:

We plan to continue these three techniques to correct various types of renovascular hypertension. A long-term follow-up of a larger number of patients is essential for final conclusions.

Publications:

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Chabner, B.A., Johnson, R.E., Chretien, P.B., Schein, P.S., Young, R.C., Canellos, G.P., Hubbard, S.H., Anderson, T., Rosenoff, S.H., and DeVita, V.T.: Percutaneous liver biopsy, peritoneoscopy and laparotomy: An assessment of relative merits in the lymphomata. Brit. J. Cancer 31 (Suppl. II):242-247, 1975.

Danforth, D.N.,Jr., and Javadpour, N.: Total unilateral renal destruction caused by irradiation for Hodgkin's disease. Urology 6:790-793, 1975.

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Felix, E.L., Cohen, M.H., Bernstein, A.D., and Schwartz, J.H.: Adult intussusception: A case report of recurrent intussusception and literature review. Amer. J. Surg.

Javadpour, N., Bartter, F.C., and Doppman, J.L.: Segmental renal vein renin assay and segmental nephrectomy for correction of hypertension. J. Urol.

July 1, 1975 to June 30, 1976

Clinical Studies in General Surgery

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG	NCI
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OTHER:	L. B. Thomas	Chief, Lab. of Pathology	LP	NCI
	W. Terry	Chief, Immunology Br.	I	NCI
	R. C. Young	Chief, Medicine Branch	M	NCI
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	M. Lippman	Senior Investigator	M	NCI
	R. E. Johnson	Chief, Rad. Oncol. Br.	RO	NCI
	J. L. Doppman	Chief, Diag. Radiol. Dept.	DR	CC

COOPERATING UNITS (if any)

Laboratory of Pathology, NCI; Immunology Branch, NCI; Medicine Branch, NCI; Laboratory of Chemical Pharmacology, NCI; Radiation Oncology Branch, NCI; Diagnostic Radiology Department, CC

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.0	6.0	3.0

SUMMARY OF WORK (200 words or less - underline Keywords)

The Surgery Branch has a variety of studies investigating innovative therapies for patients with malignant disease. The major emphasis of these studies is in the treatment of malignant melanoma, soft tissue sarcomas, and osteogenic sarcomas. New protocols on the use of methotrexate in head and neck cancer and in the treatment of rectal cancer have begun in the past year. The major emphasis in Surgery Branch cancer therapy is in adjunctive therapy with emphasis on the use of immunotherapy as a surgical adjuvant.

Project Description:

Part I. Surgical Treatment of Malignant Melanoma

Part a. Natural History

Part b. Immunotherapy and Chemotherapy as Postsurgical Adjuvants in the Treatment of Malignant Melanoma

Part c. Evaluation of Staging Laparotomy

Part d. Studies on Intralesional Treatment of Cutaneous Recurrences

Part e. Intralesional BCG as a Treatment for Patients with a Primary Malignant Melanoma

Part f. An Investigation of Human Melanoma for the Presence of Steroid Hormone Receptors

Part II. Surgical Treatment of Sarcomas

Part a. Retrospective Sarcoma Study

Part b. Improved Treatment of Soft Tissue Sarcomas of the Extremities, Head and Neck and Trunk

Part c. Osteogenic Sarcomas

Part d. Epidemiologic Studies

Part e. Rehabilitation Studies

Part III. The Use of Methotrexate as a Preoperative Adjuvant in Patients with Head and Neck Squamous Carcinomas

Part IV. Breast

Part a. Early Breast Cancer

Part b. Treatment of Primary Breast Cancer

Part c. Treatment of Metastatic Breast Cancer

Part d. Treatment of Male Breast Cancer

Part V. Genitourinary Aspects of Cancer Management

Part a. Management of Massive Bladder Hemorrhage Secondary to Cyclophosphamide or Irradiation

Part b. Tumor Markers in Germ Cell Tumors of the Testis

Part c. Localization of HCG and AFP in Germ Cell Tumors of the Testis

Part VI. Endoscopy

Part a. Laparoscopy

Part b. Colonoscopy

Part VII. Computer Applications to Research and Clinical Problems in the Surgery Branch

Part a. Serum and Lymphocyte Inventory

Part b. Immune Evaluation Visit

Part c. Randomized Protocols

Part d. Nutrition Studies

Part VIII. Surgical Treatment of Rectal Cancer

Part IX. The Effects of Thymosin in Patients with Carcinomas of the Lung and Esophagus Treated by Radiation Therapy

Part X. The Assessment of Pulmonary Nodules in Patients with Extrathoracic Malignancy

Part XI. Nutritional Problems of the Cancer Patient

Project Description:

Part I. Surgical Treatment of Malignant Melanoma

Part a. Natural History

Objectives:

To review the previous experience in this Branch with the surgical treatment of malignant melanoma in order to determine guidelines for future therapy. We have concentrated particularly on correlations between Clark's level of invasion of the primary melanoma and the incidence of lymph node metastases in palpable and nonpalpable regional lymph nodes in patients undergoing lymphadenectomy.

Methods Employed:

Patients seen in the NCI Surgery Branch with melanoma had their slides of the primary lesion reviewed by members of the NIH Pathology Department. An

attempt was made to determine the level of invasion on each primary tumor. Cases in which no primary tumor was available, or in which sections were felt to be inadequate, were eliminated. An attempt was made to consider only operable cases; therefore, cases with disseminated disease or satellitosis were excluded, and only patients undergoing prophylactic or therapeutic lymph node dissections were considered. The hospital record of all patients was reviewed to determine whether or not the patient had palpable lymph nodes prior to surgery, the type of surgery that was performed, the microscopic findings in the lymph nodes, and the ultimate outcome of each of the 117 qualifying patients.

Major Findings:

Important prognostic factors included the site of the melanoma, the number of lymph nodes pathologically containing tumor, the clinical positivity or negativity of the regional lymph nodes preoperatively, the sex of the patient, the level of invasion of the primary lesion, and the thickness of the primary lesion. The first three factors appeared to correlate more strongly with prognosis than the latter three factors. The site of the primary melanoma and the histological status of the lymph nodes were correlated with each other as there was a higher degree of nodal positivity in patients with primary melanomas on the extremities. In addition, the site of the melanoma and the histological status of the lymph nodes appeared to exert independent effects on prognosis, beside being correlated with each other. The preoperative clinical assessment of the lymph nodes was highly correlated with the subsequent pathological findings, and had no additional prognostic value except, possibly, in patients with 1-3 positive lymph nodes, in whom the prognosis was (non-statistically) decreased in patients with clinically positive lymph nodes as compared to patients with clinically negative lymph nodes.

The sex of the patient, the level of invasion of the primary lesion, and the thickness of the primary melanoma were also correlated with prognosis, although less strongly than the first three factors mentioned above. The better prognosis in female patients appeared to be due to the increased association of extremity melanomas (as opposed to truncal or head and neck melanomas) in the female patients. There was no additional independent prognostic value in being female. The level of invasion and the thickness of the melanoma were correlated with each other and the deeper lesions and the thicker lesions were found to have an increased incidence of lymph node metastases as compared to melanomas that were invasive to more superficial levels or melanomas that were thinner. Once the histological pattern of the dissected lymph nodes was known, knowledge of the level of invasion or the thickness of the melanoma had additional prognostic value only in patients with histologically negative lymph nodes.

The survival of patients with 1-3 positive nodes was significantly better than survival of patients with 4 or more positive lymph nodes. In addition, those patients with positive lymph nodes showed correlations between level of invasion (or thickness) and numbers of positive nodes.

Significance to Cancer Research and the Program of the Institute:

It is hoped that this additional information may be of help in deciding which patients, if any, should be subjected to prophylactic lymph node dissection, and in estimating prognosis for potential candidates for therapy.

Proposed Course:

We plan to complete this study as outlined and use it as one of the bases for evaluation of the Surgery Branch approach to the treatment of malignant melanoma.

Project Description:

Part b. Immunotherapy and Chemotherapy as Postsurgical Adjuvants in the Treatment of Malignant Melanoma

Objectives:

The purpose of this study is to evaluate in a prospective randomized protocol the value of chemotherapy and immunotherapy in patients with Stage II malignant melanoma following local excision and regional lymph node dissection.

Methods Employed:

This protocol is being conducted in conjunction with the Medical Oncology and Immunology Branches. Eligible patients include those with Level IV melanomas greater than 2.25 mm thick, Level V lesions, all pathologic Stage II patients, and all patients with local recurrence of melanoma within 5 cm of the original primary melanoma. All patients receive wide local excision and regional lymph node dissection. Patients are then randomized to one of four arms. Patients randomized to observation alone are followed in the Surgery Branch. Patients randomized to chemotherapy receive 200 mg/m^2 of methyl-CCNU every six weeks. Patients randomized to receive BCG or BCG plus immunization with allogeneic neuraminidase-treated cells are followed in the Immunology Branch. Immunologic evaluation of patients is performed serially. To date, approximately 40 patients have been entered into the above protocol. The Surgery Branch performs excisions and lymph node dissections on about one-half of all patients entering this protocol. Immunologic evaluation has revealed that the initial immunization schedule with neuraminidase-treated cells is producing allogeneic immunity as measured by *in vitro* seriologic and cell-mediated immune assays.

Significance to Cancer Research and the Program of the Institute:

This protocol is designed to evaluate the role of immunotherapy and chemotherapy in the treatment of patients with malignant melanoma. These postoperative adjuvant treatments may improve the poor prognosis of patients with this disease.

Proposed Course:

This study is being continued.

Project Description:

Part c. Evaluation of Staging Laparotomy

Objectives:

The objectives of this study are (1) to determine the incidence of hepatic or para-aortic melanoma involvement in patients who have disease confined to the inguinal lymph nodes, and (2) to determine the incidence of deep inguinal lymph node positivity in patients with varying degrees of positivity in the superficial inguinal lymph nodes.

Methods Employed:

Twenty-six consecutive patients with malignant melanoma of the lower extremities and documented metastases to the superficial groin, without evidence of other dissemination, were studied. Each patient received multiple radioisotope and chemical studies to assess tumor dissemination. No patient had a positive liver scan. Each patient was then subjected to laparotomy. The liver was carefully palpated and/or biopsied, as were the para-aortic lymph nodes. If melanoma was found in either of these locations, the operative procedure was terminated and the patient was placed on a chemotherapy-immunotherapy protocol. If this examination of liver and para-aortic lymph nodes was negative, then the patient, during the same operation, underwent dissection of the deep inguinal lymph nodes, since each of these patients had documented metastases to the superficial inguinal lymph nodes.

Major Findings:

Three of 26 patients had positive liver or para-aortic lymph node biopsies at the time of surgery (Table 10). Two of these patients had massive local disease in the superficial groin and one of the three patients had a local recurrence at the operative site at the ankle.

Twenty-three of the 26 patients underwent ipsilateral iliac and obturator lymph node dissection, which proved positive in 3/23 patients (13%). Patients with only microscopic superficial inguinal involvement had no hepatic or nodal spread noted at laparotomy.

Significance to Cancer Research and the Program of the Institute:

These studies indicate that patients with bulky nodal disease have, in this series, a better chance of having hepatic or para-aortic tumor dissemination compared to patients with less bulky disease. Patients with only microscopic positivity in the superficial groin had no deep iliac lymph node involvement,

Table 10

Clinical Stage Prior to Positive Superficial Groin Dissection	Incidence of Liver or Para-Aortic Node Positivity	Incidence of Positivity in Dissected Deep Groin Nodes*
I	0/5 (0%)	0/5 (0%)
II	3/21 (14%)	3/18 (17%)
TOTAL	3/26 (12%)	3/23 (13%)

*Does not include the three patients with liver or para-aortic node positivity since no deep groin dissection was performed in these patients.

hepatic involvement, or para-aortic involvement. Patients with clinically positive superficial inguinal lymph nodes had an 18% incidence of positive deep inguinal node dissection.

Proposed Course:

This information will be of value in reassessing the approach of the Surgery Branch to the surgical treatment of melanoma. Of particular interest is the group of patients with only microscopic positivity in the superficial groin. As more patients of this type are studied it may be found to be unnecessary to perform deep inguinal dissection in this group.

Project Description:

Part d. Studies on Intralesional Treatment of Cutaneous Recurrences

Objectives:

To evaluate the role of BCG, DNCB, and other agents in the intralesional treatment of malignant melanoma.

Methods Employed:

In an initial nonrandomized study on five patients, 82 lesions were injected with BCG (10^7 Tice organisms per injection), 179 lesions were injected with DNCB (50 micrograms), 35 lesions were injected with a combination of BCG and DNCB, 43 lesions were injected with acetone, the diluent for dinitrochlorobenzene, and 59 lesions were inoculated with either saline, water, or other diluents. In a second study, 12 patients received only intralesional BCG or only intralesional DNCB.

Major Findings:

Both agents were able to destroy over 90% of the injected intradermal nodules, although DNCB required two injections more frequently than did BCG in order to destroy individual nodules. Intradermal disease was more easily obliterated than subcutaneous disease with intralesional treatment with either agent. Four of six patients in each group represent treatment failures at the present time. The two "successes" in the BCG group are without evidence of disease 16 and 17 months respectively from the beginning of intralesional treatment. In the DNCB group one patient is without evidence of disease five months after beginning of treatment and one patient with subcutaneous disease is stable, ten months after beginning of treatment. The other eight patients in the study (four in each group) are considered treatment failures. Four patients treated with intralesional DNCB developed distant metastases between five and seven months after beginning of treatment. Two patients in the intralesional BCG group developed distant metastases four and six months after beginning of treatment, and two had uncontrolled progressive local subcutaneous disease.

Significance to Cancer Research and the Program of the Institute:

Throughout our studies, we have been impressed by the close correlation between local destruction by the injected agent, and the ability of that agent to destroy lesions. Superficial lesions are also more easily eradicated than deep subcutaneous lesions by any agent. For this reason, we have hypothesized that the major beneficial effect of intralesional inoculation of multiple cutaneous metastases is local destruction by the injected agents. We have been impressed with the ability of a nonreplicating agent to give equivalent regression rates and to produce the same in vitro change as is observed after injection of BCG, a living agent, associated with a high incidence of side effects. If DNCB is capable of producing the same in vitro changes as BCG, then it is also possible that the chemical agent might be substituted for BCG in studies on systemic immunotherapy as an adjuvant to treatment of solid tumors.

Proposed Course:

If the nonreplicating chemical agent DNCB continues to be as effective as BCG in intralesional treatment of melanoma nodules, then we feel that DNCB should be used for this purpose, because of the dramatic difference in morbidity and complication rates following injection of DNCB. Although BCG is regularly associated with fever and malaise, we have observed no adverse effects of DNCB inoculation, except local ulceration, which occurs following either agent's intralesional use. Finally, if DNCB has the same systemic effect, then it may be the agent of choice instead of replicating agents for systemic immunotherapy. These hypotheses would have to be tested in randomized trials.

Project Description:

Part e. Intralesional BCG as a Treatment for Patients with a Primary Malignant Melanoma

Objectives:

This study is designed to determine whether treatment with intralesional BCG is capable of improving the prognosis of patients with primary malignant melanoma.

Methods Employed:

All patients having primary malignant melanomas have either punch or incisional biopsy. Those patients that have Clark's Level IV or V malignant melanoma are entered into a prospective, randomized trial that contains two treatment arms. The first treatment arm consists of immediate wide local excision with regional lymph node dissection. In the second treatment arm patients receive intralesional BCG followed two to four weeks later by wide local excision and regional lymph node dissection. Patients are then followed both clinically and with serial immunologic studies. To date, approximately

31 patients with primary malignant melanoma have been seen in the Surgery Branch and 11 patients have been entered into this treatment protocol. Six have received intralesional BCG injection and five patients have received immediate surgery.

Major Findings:

Of the 11 patients that have entered into this protocol, none has yet recurred. Of the six patients receiving intralesional BCG, five have completed their full course of injection and surgery. Of these five patients, the intralesional BCG eliminated all evidence of local tumor in three patients. In the five patients receiving BCG injection who have had their lymph node dissections, none have had tumor present in draining regional nodes. Data are too preliminary to draw conclusions regarding the clinical effectiveness of BCG treatment.

Significance to Cancer Treatment and the Program of the Institute:

This study is designed to improve the prognosis and survival of patients with poor prognosis primary malignant melanoma using immunotherapy with intralesional BCG.

Proposed Course:

This study is being continued.

Project Description:

Part f. An Investigation of Human Melanoma for the Presence of Steroid Hormone Receptors

Objectives:

This study was designed to investigate the possibility that human melanoma contains steroid hormone receptor proteins; patients with estrogen receptors who have failed conventional therapy will be treated with additive estrogen therapy to determine the significance of the receptor protein.

Methods Employed:

Patients with melanoma have a portion of their tumor assayed for steriod hormone receptors using a specific competitive protein-binding assay. Pathologic control is obtained on all tumor samples.

Major Findings:

Specific estrogen binding was present in 9 of 22 patients; androgen receptor was demonstrated in 4 of 18 patients, progesterone receptor in 4 of 15 patients, and glucocorticoid receptor in 3 of 15 patients.

Significance to Cancer Research and the Program of the Institute:

The biologic behavior of melanoma suggests that there may be a subset of patients with a hormonally responsive tumor. This study has demonstrated that specific steroid hormone receptors are present in human melanoma. It is too early to state if the use of hormone receptor analyses will be useful in selecting a hormonally responsive subset of patients.

Proposed Course:

A larger number of melanoma tumor samples must be assayed to determine the incidence of receptor positivity and to make clinical correlations. Additional patients must be treated with hormonal therapy to determine its efficacy.

Project Description:

Part II. Surgical Treatment of Sarcomas

Part a. Retrospective Sarcoma Study

Objectives:

The objectives of this project were to review, verify, update, and analyze information on patients treated for sarcomas by the Surgery Branch from 1954 through 1974.

Methods Employed:

(1) Information on sarcoma patients had been initially collected and stored using the data forms and system developed in collaboration with the Division of Computer Research and Technology.
(2) During the past year the patient records were reviewed and the computer data file was corrected and brought up-to-date through December 1974.
(3) Corrected information was entered using WYLBUR.
(4) The tape master file was updated primarily by using the editing and updating programs generated by the DCRT Data Management System. Several additional special updates were written using IRS.
(5) Retrieval, display, and analysis of the sarcoma data was accomplished with IRS and a survival/recurrence/plot program developed by the Biostatistical Information Systems Unit.

Major Findings:

In this study 284 patients were classified into four categories - patients who:

(1) Received definitive surgical treatment for primary or persistent disease with no metastasis.

(2) Received definitive surgical treatment for locally recurrent disease with no metastasis.
(3) Presented with metastatic disease.
(4) Presented with primary or persistent disease with no metastasis who received no treatment or treatment other than surgery.

Table 11 shows the distribution of patients by category and histologic diagnosis. Survival and recurrence data are currently being analyzed.

Significance to Cancer Research and the Program of the Institute:

This information constitutes a historical control for comparison with that obtained from the randomized protocols.

Proposed Course:

Analysis of the information will continue.

Project Description:

Part b. Improved Treatment of Soft Tissue Sarcomas of the Extremities, Head and Neck and Trunk

Objectives:

To attempt to improve the treatment of patients with soft tissue sarcomas. In the treatment of soft tissue sarcomas of the extremities, the value of radical surgery, chemotherapy and immunotherapy are being evaluated. In the treatment of soft tissue sarcomas of the head and neck and trunk, the value of postoperative adjuvant chemo- and immunotherapy are being evaluated.

Methods Employed:

All patients with soft tissue sarcomas of the extremities with no evidence of metastatic disease are being randomized to one of three treatment arms. In one treatment arm, patients receive standard radical excisional surgery plus postoperative chemotherapy. In the second treatment arm, patients receive conservative surgery followed by high-dose radiation therapy and postoperative adjuvant chemotherapy. In the third treatment arm, patients receive conservative surgery, high-dose radiation therapy and postoperative chemotherapy and immunotherapy. Chemotherapy in all arms consists of adriamycin and cytoxan. Immunotherapy consists of the intravenous injection of Corynebacterium parvum.

Patients having soft tissue sarcomas of the head and neck or trunk without evidence of distant metastases are randomized to a treatment protocol consisting of two arms. One treatment arm receives surgery and adjuvant chemotherapy with adriamycin and cytoxan. The second treatment arm consists of surgery followed by adjuvant chemotherapy and immunotherapy.

Table 11

SURGERY BRANCH EXPERIENCE WITH SOFT TISSUE AND BONY SARCOMAS

	(1)	(2)	(3)	(4)	Total
Fibrosarcoma	12	11	9	3	35
Liposarcoma	13	4	1	6	24
Osteosarcoma	32	2	21	6	61
Parosteal sarcoma	3				3
Chondrosarcoma	16	4	8	13	41
Neurofibrosarcoma	1	1			2
Leiomyosarcoma	3	1	3	2	9
Rhabdomyosarcoma	19	7	8	8	42
Mesenchymoma	3	2	1	2	8
Synovial cell sarcoma	3	2	8	2	15
Undifferentiated sarcoma	10	4	4	6	24
Other sarcoma	9	3	6	2	20
Total	124	41	69	50	284

All patients are extensively evaluated both clinically and in terms of immunologic competence. Patients are followed postoperatively with serial monitoring of immunologic, as well as clinical status.

Major Findings:

To date, 18 patients have been randomized in these treatment protocols. Thus far, there have been no recurrences in any of the treatment arms. By comparison with the extensive Surgery Branch historical experience of 284 patients with soft tissue sarcomas, the potential value of adjuvant immunotherapy will be determined. Of interest is the fact that most patients receiving radiotherapy, immunotherapy and chemotherapy have tolerated this concomitant combination well. Only two patients have experienced local reactions to the radiotherapy and have required delay of chemotherapy administration. The data are too preliminary to draw conclusions regarding therapeutic effectiveness of any of the treatment arms.

Significance to Cancer Research and the Program of the Institute:

This study is designed to improve the poor prognosis of patients with soft tissue sarcomas.

Proposed Course:

This study is being continued.

Project Description:

Part c. Osteogenic Sarcomas

Objectives:

This clinical protocol is designed to test the effectiveness of adjuvant chemotherapy and immunotherapy in the postoperative treatment of patients with osteogenic sarcoma.

Methods Employed:

All patients with osteogenic sarcomas without evidence of distant metastatic spread receive amputations in the Surgery Branch to achieve local control of their tumors. All patients then receive high-dose methotrexate with citrovorum rescue. This chemotherapy begins at 50 mg/kg and escalates to 250 mg/kg as tolerated. All patients are randomized to one of two treatment arms. One treatment arm receives high-dose methotrexate alone. The second group receives high-dose methotrexate plus weekly injections of BCG by Heaf gun scarification. All patients are extensively studied both clinically and in terms of their immunologic competence preoperatively and serially in the postoperative period.

Major Findings:

To date, 21 patients have been entered into this treatment protocol. The data are too preliminary to draw any conclusions regarding the relative effectiveness of BCG as a postoperative adjuvant.

Significance to Cancer Research and the Program of the Institute:

This study is designed to improve the treatment of patients with osteogenic sarcoma.

Proposed Course:

This study is continuing.

Project Description:

Part d. Epidemiologic Studies

Objectives:

In collaboration with the Epidemiology Branch of the National Cancer Institute special effort is being exerted towards the epidemiologic study of patients with bone and soft part sarcoma. Initial efforts have been directed toward a retrospective study of 284 patients who have undergone treatment at the Clinical Center since 1953. It is hoped that these studies will uncover etiologic associations and risk factors that will be of importance in understanding the occurrence of this highly malignant, but relatively rare group of tumors.

Methods Employed:

A computerized data set had been developed to identify all patients in this group along with major demographic features of each patient. This information served as a basis for pulling charts on patients in the group. All charts were reviewed for specific information concerning incidence of cancer in the families of patients, specific etiologic factors such as chemical exposures or radiotherapy and the occurrence of multiple cancers within the patient group.

Major Findings:

Three interesting associations that will serve as the basis for case reports with literature review have been uncovered through these studies. First is a case of osteogenic sarcoma of the humerus developing 23 years after injection of thoratrast. Second is a case of fibrosarcoma developing in the muscle of a patient with myotonic dystrophy and thirdly, there is a case of fibrosarcoma developing in a patient with von Recklinghausen's disease. In addition to these specific associations, 11 patients with sarcoma were noted

subsequently to develop carcinoma. The frequency of this occurrence is thought to be many times the expected occurrence and will be the subject for continued investigation. Additionally, some association between fibrosarcoma and prior radiotherapy was noted and this too will be investigated.

Significance to Cancer Research and the Program of the Institute:

The findings of these epidemiologic studies will serve to confirm previous observations in the literature and will serve as a basis for developing new associations and risk factors. These observations should be of importance in uncovering direct causal relationships and in developing methods for control.

Proposed Course:

Current studies will be expanded by means of prospective studies on new patients admitted to the Clinical Center. Interview techniques will be developed to obtain specific information from all patients entering with a diagnosis of sarcoma as well as from old patients returning for follow-up examination. Following these pilot studies, further epidemiologic investigations using control groups for comparison will be considered.

Project Description:

Part e. Rehabilitation Studies

Objectives:

To investigate methods of improving the total course of rehabilitation in patients receiving amputations as major ablative surgery for osteogenic sarcoma of the lower extremities. To diminish the time required between surgery and functional use of a permanent prosthesis and to increase the quality and capacity of ambulation in all stages throughout the rehabilitation period.

Methods Employed:

Patients receiving above-the-knee amputations for osteosarcoma were chosen for investigation. A thorough review of the experience of major rehabilitation centers throughout the world was made. From this review and from preliminary experience at the NCI, rigid dressings (composed of plaster of paris) have been chosen as the best type of dressing for all stumps. Unclear, however, was whether immediate ambulation (24 hours post-surgery) or delayed but early ambulation (10 days to 2 weeks post-surgery at the time of suture removal) was superior. Also unclear was whether the necessary hardware for ambulation need be prepared by a prosthetist or could be prepared by a physical therapist.

Therefore, prior to surgery, patients have been prospectively randomized to receiving either a prosthesis that allows for immediate ambulation or for delayed ambulation. They have at the same time been randomized to have the prosthesis prepared by a prosthetist or by members of the Department of Physical Medicine at the NIH. An intense and thoughtful program of rehabilitation has been devised allowing for reasonably rigid comparison of multiple aspects of rehabilitation among the various groups.

Major Findings:

These studies are just beginning. Patients were first admitted to the protocol January 1976.

Significance to Cancer Research and the Program of the Institute:

The prognosis for prolonged survival in osteogenic sarcoma is still dismal. Still we are obligated to provide in those patients receiving major amputation for tumors the best chance for as normal, functional, and productive a life as is possible. The rehabilitation of patients who receive such amputations is, therefore, a vital part of the total care of patients treated at this Institute and is, therefore, a significant part of research relating to the patient and his tumor.

Proposed Course:

Continued research in rehabilitation of the amputee patient with cancer as outlined above. Possible extension of protocols to patients with other types of amputations. Continued provision of excellent quality prostheses for all eligible amputees and of programs for total rehabilitation of the amputee patient.

Project Description:

Part III. The Use of Methotrexate as a Preoperative Adjuvant in Patients with Head and Neck Squamous Carcinomas

Objectives:

To derive a high dose methotrexate regimen to be used preoperatively in patients with clinically operable head and neck squamous carcinomas.

Methods Employed:

As an initial determination of the feasibility of the use of high dose methotrexate as a preoperative adjuvant, a dose regimen consisting of methotrexate 240 mg/m^2 I.V. over a 24 hour period followed by leucovorin rescue was administered on day 1 and day 7, with operation being performed on day 14-16. The patients were observed carefully for evidence of methotrexate toxicity by laboratory and clinical assessments during operation and in the postoperative

period. After evaluation of this dose regimen in 30 patients, the 24 hour infusions were escalated to 500 mg/m^2 in three patients, then to 1000 mg/m^2 in three patients. In each instance two infusions were given prior to operation.

Major Findings:

In the 30 patients who received 240 mg/m^2 methotrexate intravenously, no clinical or laboratory evidence of toxicity was evident when the patients were compared with a matched group of 30 patients with similar tumors who received similar surgical treatment. Reduction in gross size of the tumor or lymph nodal metastases was determined in 23 (77%). Life table analysis of the postoperative interval before death of the methotrexate-treated patients who have died did not differ from that of the controls treated by surgery alone. However, among patients who developed recurrences there was a statistically increased interval ($p < .05$) before tumor recurrence in the patients who received adjuvant methotrexate. In the patients receiving methotrexate 500 mg/m^2 and 1000 mg/m^2 I.V., reduction in tumor size occurred in all prior to operation. Pathologic examination of a hemiglossectomy specimen that contained the site of a 4 cm diameter tumor prior to treatment failed to reveal residual tumor cells. Blood levels of methotrexate in two patients receiving the 1000 mg/m^2 dose were in the 10^{-5} molar range during the period of infusion.

Significance to Cancer Research and the Program of the Institute:

This study confirms previous observations of the responsiveness of head and neck squamous carcinoma to methotrexate. Furthermore, it demonstrates that the drug can be given in a high dose regimen preoperatively without morbidity. Because of the relatively low cure rates achieved with radiation therapy and surgery in the treatment of Stage III and IV head and neck squamous carcinomas, and the failure of these two modalities in combination to further increase cure rates, the results of this preliminary investigation provide a rationale for the determination of the effects on cure rates of high doses of methotrexate, and methotrexate in combination with other drugs given preoperatively. In patients whose tumors respond to such regimens, a determination of the effect on cure rates of additional high dose adjuvant chemotherapy given postoperatively would also be warranted.

Proposed Course:

The data thus far accumulated will be used to derive an intravenous methotrexate infusion of 36 hours duration which will maintain a blood methotrexate infusion of 36 hours duration which will maintain a blood methotrexate level of 10^{-5} molar. This dose regimen will then form the basis of a prospective randomized protocol that will compare the effects on tumor control of this regimen given preoperatively with the results after surgery alone for tumors of the oral cavity.

Project Description:

Part IV. Breast

Part a. Early Breast Cancer

Objectives:

In the management of breast cancer, improvement in survival and mortality rates will come about through early detection at a time when nodal metastasis and widespread dissemination are least likely to be present. To this end, several diagnostic procedures are being evaluated in the breast problem clinic of the Surgery Branch. Patients with breast masses, nipple discharge, or abnormal breast x-rays are accepted for evaluation.

Methods Employed:

In addition to routine history and physical examination, particular emphasis is put on the detection of breast cancer by means of xerography and needle aspiration of breast masses.

Major Findings:

As in other concerted studies of breast disease, patients with well defined masses present relatively little problem in diagnosis since the mass may be rapidly deflated by aspiration if it is cystic or may be amenable to direct biopsy for evaluation. On the other hand, those patients with nonpalpable lesions detected only through radiographic means present major problems in finding the lesion for diagnosis. Blood and urinary marker studies and isotope scanning of the breast formerly under evaluation have been discontinued. These methods were found not to be helpful in the group of patients with nonpalpable lesions detected only by x-ray examination.

Significance to Cancer Research and the Program of the Institute:

Continued efforts to diagnosis breast cancer at an early stage should improve the survival and mortality rates of patients who otherwise may not have been detected until overt clinical signs were present. Furthermore, information obtained through these studies should lead to a better understanding of the natural biology of breast cancer.

Proposed Course:

Continued effort will be exerted to detect nonpalpable lesions aided in the future by specimen radiography using a specialized instrument known as a Facitron. This instrument should be of great help in detecting calcium markers in specimens containing no palpable mass.

Project Description:

Part b. Treatment of Primary Breast Cancer

Objectives:

Evaluation of postoperative chemotherapy and immunotherapy in the treatment of patients with cancer of the breast, metastatic to axillary lymph nodes, following mastectomy and axillary dissection.

Methods Employed:

The Surgery and Medicine Branches are collaborating in Protocol BTS-135, Evaluation of a Combined Modality Approach to the Therapy of Mammary Carcinoma in Patients with T_1, 2, 3A, and 4B with N(+) Lesions. This is the basic protocol for adjuvant chemotherapy of surgically treated patients who have positive axillary nodes. Such patients are currently being randomized between chemotherapy with cytoxan, methotrexate and 5FU (CMF), vs. CMF with C. parvum, vs. observation (previously) or L-PAM (currently).

Major Findings:

Thirty-seven patients have thus far been entered on these studies with randomization of 18 to receive L-PAM or observation, 16 to the CMF arm, and 3 to the immunotherapy plus CMF arm.

The lowest PHA stimulation values were found in the group treated with cytoxan, methotrexate and 5-Fluorouracil alone (Arm B), although none of the differences observed between the groups was stastistically significant. In Arm B, the average PHA stimulation index (± SEM) on 12 samples taken during treatment was 46 ± 11. Arm C (CMF + C. parvum) had a mean stimulation index of 49 ± 15, and Arm A (observation and L-PAM) had a mean stimulation index of 50 ± 6.

Limited testing of cell-mediated cytotoxicity to long-term cultured breast cancer lines revealed no differences between the CMF arm and the observation/L-PAM arm.

Significance to Cancer Research and the Program of the Institute:

The aim of the multidisciplinary approach to the treatment of breast cancer is the appraisal of current treatment modalities and their improvement by the use of adjuvant techniques. Also the procurement of material for biologic assay will lead to a better understanding of the nature of breast cancer.

Proposed Course:

These studies will be continued.

Project Description:

Part c. Treatment of Metastatic Breast Cancer

Objectives:

To improve the treatment and diagnostic accuracy of predicted assays for patients with metastatic cancer and to develop more reliable methods of identifying those patients who may be helped by endocrine ablation.

Methods Employed:

The Surgery Branch has aided the Medicine Branch on a series of interlocking treatment protocols that have included palliative resections, endocrine ablation, and chemotherapy. These are illustrated by (1) BTS-122, A Study to Evaluate the Effect of Cyclophosphamide, Methotrexate, and 5-Fluorouracil vs. Cyclophosphamide, Adriamycin, and 5-Fluorouracil. This study was designed to evaluate eligible patients who have not received previous chemotherapy but who present with, or are referred with, metastatic breast cancer. The Surgery Branch has been involved in the overall evaluation of these patients and obtaining materials necessary for histologic examination and determination of endocrine receptor status of malignant tissues and corresponding normal tissues. This study itself is not entering new patients at this time, but accumulating results on the patients already admitted. (2) BTS-123, A Study to Evaluate the Effect of Oophorectomy with and without Adjuvant Chemotherapy in the Management of Recurrent Mammary Carcinoma in Premenopausal Women. In the past year 9 patients have been entered into this study. These patients have had their oophorectomy performed by the Surgery Branch. (3) Protocol BTS-124, A Study Designed to Evaluate the Effect of Estrogen Therapy with and without Additive Chemotherapy in the Management of Recurrent Mammary Carcinoma in the Postmenopausal Female. Nineteen new patients have been added to this study and though no direct endocrine ablative procedures have been performed on these patients, there have been well in excess of 125 biopsies provided for study by the Medicine Branch laboratory involved with determination of multiple endocrine receptor proteins. (4) Protocol B-151, The Prospective Use of Steroid Hormone Receptor in Predicting the Response to Endocrine Manipulation in Metastatic Breast Cancer, has only just been finally approved and activated. No patients have been formally entered on this study though five adrenalectomies have been performed by members of the Surgery Branch for patients who have had their tumors studied for receptor proteins in a manner comparable to that which is expected in Protocol B-151.

Major Findings:

These studies are in the process of accumulating data.

Significance to Cancer Research and the Program of the Institute:

The information being accumulated will allow the application of the most appropriate therapy to the individual patient with metastatic breast cancer

based on a better understanding of the individual biologic characteristics of the tumor. New treatment modalities are being evaluated in an attempt to prolong survival of patients with metastatic breast cancer.

Proposed Course:

It is planned to expand and further develop currently instituted protocols and studies with particular emphasis on the development of new protocols within the Surgery Branch.

Project Description:

Part d. Treatment of Male Breast Cancer

Objectives:

The purpose of these studies is to evaluate the steroid hormone receptor status, serum and urine hormone alterations, and perform clinical correlations on male patients with advanced breast cancer prior to and subsequent to endocrine ablation.

Methods Employed:

Patients with metastatic or recurrent tumor have samples assayed for steroid hormone receptors and then undergo bilateral orchiectomy and, upon failure, subsequent adrenalectomy. Urine and serum hormonal alterations are correlated with receptor activity in the tumor and with response to therapy.

Major Findings:

Most male breast cancers contain steroid hormone receptors. Six patients have been treated over the past year at the NCI; five have had hormonal receptor in their tumor. The number of patients is, therefore, still too small to produce significant clinical correlates.

Significance to Cancer Research and the Program of the Institute:

This study is evaluating the value of steroid hormone receptor analyses to select those patients who will respond to endocrine manipulations. Evaluating serum and urine hormonal manipulations may allow early identification of tumor recurrence.

Proposed Course:

This study remains in its early stages with relatively brief periods of follow-up. Additional patients must be added to this study for significant correlations to be obtained.

Project Description:

Part V. Genitourinary Aspects of Cancer Management

Part a. Management of Massive Bladder Hemorrhage Secondary to Cyclophosphamide or Irradiation

Objectives:

Severe acute hemorrhagic cystitis following administration of cyclophosphamide or radiation therapy for cancer has been relatively common among patients in the National Cancer Institute. Bleeding is occasionally massive and life-threatening, and despite the frequent use of multiple transfusions and other conventional modalities of treatment, the morbidity and mortality have been substantial. The purpose of this study is to evaluate the efficacy of local instillation of refined formaldehyde into the bladder to treat massive bladder hemorrhage.

Methods Employed:

The safety and efficacy of formaldehyde instillation into the bladder was first evaluated in animals. It was then applied to 9 patients with life-threatening hemorrhage refractory to conventional treatment.

Major Findings:

Cyclophosphamide induced the following changes in the bladder of man and experimental animals: (a) atypia of transitional cells with ulceration of the mucosa, (b) massive submucosal telangiectasia, (c) fibrosis of the detrusor muscle leading to diminished function of the bladder. These changes have been observed in patients suffering from cyclophosphamide cystitis, as well as radiation cystitis, and have been observed in mice and dogs experimentally. On the other hand, formaldehyde instillation into normal bladders of mice results in no significant changes detectable by biopsy. We have successfully utilized intravesical instillation of 200 ml of 10% refined formaldehyde in 9 patients with recurrent hematuria refractory to conventional therapy. Follow-up of these patients over 1-2 years has revealed no undesirable side effects. Two patients required a second instillation of formaldehyde.

Significance to Cancer Research and the Program of the Institute:

These studies have demonstrated an alternative therapy for intractable radiation or drug-induced cystitis with massive bleeding. This alternate modality of treatment does not require mutilating radical surgery which would leave the patient with a urinary diversion and loss of the bladder. The lack of complications of refined formaldehyde instillation and the effectiveness of hemostasis achieved have encouraged us to continue this program.

Proposed Course:

We plan to continue this study and follow the patients who have received this treatment with periodic bladder biopsy, cystometrogram, and renal function studies, to evaluate the long-term safety and effectiveness of this modality of treatment. A larger number of patients will be required for a better evaluation of the effectiveness and complications of intravesical instillation of refined formaldehyde.

Project Description:

Part b. Tumor Markers in Germ Cell Tumors of the Testis

Objectives:

Germ cell tumors of the testis have been shown to produce a variety of glycoproteins, including human chorionic gonadotropin (HCG), alpha fetoprotein (AFP), and carcinoembryonic antigen (CEA). The purpose of this study was to determine how frequently these tumor markers could be found in elevated levels in the peripheral circulation in patients with localized or regional disease; to assess the significance for prognosis of elevated markers prior to therapy; and to determine the value of serial marker assays as indicators of persistent or recurrent tumor after ablative therapy.

Methods Employed:

Serum samples obtained at regular intervals (usually weekly) on all patients were assayed for the beta subunit of HCG and for AFP by Dr. Thomas Waldmann, Metabolism Branch, NCI. CEA assays were performed on plasma samples by Hoffmann-LaRoche Laboratories as part of a collaborative study. Clinical data were obtained on 57 patients with clinical Stage I or II germ cell tumors of the testis at the Surgery Branch, NCI, and other cooperating institutions. Patients have been followed for 1-30 months after therapy (mean 12.5 months).

Major Findings:

(1) Seminoma - HCG was elevated in only 2 of 21 patients, both with regional nodal metastases. AFP was never elevated in these patients. CEA was elevated in 4 of 7 but there was a poor correlation with the response to therapy, so that CEA was not an accurate marker of seminoma.

(2) Non-seminoma - patients with embryonal carcinoma and teratocarcinoma frequently had elevated HCG and AFP levels (Table 12). Again, CEA was elevated in 1/3 of patients but correlated poorly with response to therapy. Taken together, HCG and AFP accurately identified the presence of tumor in 68% of these patients. There were no false positives, but 32% of patients with documented tumor present did not have HCG or AFP elevations (false negatives). There was a striking tendency for patients with elevated HCG or AFP prior to therapy to have early recurrence. Among patients with clinical Stage I

Table 12

CORRELATION OF PRETREATMENT MARKERS WITH CLINICAL STAGE

(The Primary Tumor Had Been Removed)

Histology	Clinical Stage	Results of Readioimmunoassays								
		HCG			AFP			CEA		
		+	-	(%+)	+	-	(%+)	+	-	(%+)
Seminoma	I	0	17	(0%)	0	17	(0%)	2	3	(40%)
	II	2	2	(50%)	0	4	(0%)	2	2	(100%)
Embryonal and Teratocarcinoma	I	5	16	(24%)	8	12	(40%)	2	4	(33%)
	II	4	2	(67%)	2	4	(33%)	1	2	(33%)

tumors, 71% (5/7) developed metastatic disease when HCG or AFP were elevated, while only 17% (2/12) did so when the markers were not elevated.

Recurrent or persistent tumor was identified solely by the presence of elevated markers months prior to the appearance of demonstrable metastases in 6 of 8 evaluable patients.

Significance to Cancer Research and the Program of the Institute:

This study has confirmed that circulating tumor markers can be found in elevated levels in the sera of most patients with active germ cell tumors of the testis. The accuracy of these assays in identifying even subclinical deposits of tumor, and the absence of false positive values, means that these assays can be a substantial aid in the early detection of testicular cancer, in providing a more accurate prognosis for these patients, and in monitoring their response to therapy.

Proposed Course:

The prospective study will continue to further define the accuracy of pretreatment marker levels in predicting recurrence and the value of serial marker determinations in detecting persistent or recurrent testicular cancer.

Project Description:

Part c. Localization of HCG and AFP in Germ Cell Tumors of the Testis

Objectives:

To identify the cells within germ cell tumors of the testis which contain the tumor markers, HCG and AFP.

Methods Employed:

HCG and AFP are found in elevated levels in the circulation of most patients with germ cell testicular tumors other than seminoma. In monitoring the serum levels, however, it is not uncommon to find patients with only one marker elevated while the other remains normal, or to find both markers elevated but only one return to normal after therapy. These discordant serum levels are felt to reflect production of these markers by different populations of cells with different growth rates and susceptibilities to treatment.

To identify the cell type responsible for production of each of these markers, we collected 29 primary or metastatic tumor specimens from 20 patients whose serum levels of HCG and AFP had been assayed at the time the tissue was obtained. Deparafinized sections were reacted with specific anti-HCG and anti-AFP antibody in an indirect immunoperoxidase assay.

Major Findings:

HCG was found only in the cytoplasm of syncytiotrophoblastic giant cells (STGC). All STGC stained for HCG, whether found in choriocarcinoma or found alone. Other tumor giant cells were seen on H&E sections in several tumors and did not react with the anti-HCG antiserum.

AFP was found in the cytoplasm of cells within areas of embryonal carcinoma, more frequently in the areas of endodermal sinus tumor.

There was a close correlation between elevated levels of each serum marker and positive tumor staining for the marker, with 12 of 17 showing HCG positive areas when the serum HCG was elevated, and 8 of 14 showing AFP positive areas when the serum AFP was elevated.

Significance to Cancer Research and the Program of the Institute:

The cells responsible for the production of HCG and for AFP in germ cell tumors of the testis were identified. These two markers localized to entirely different cell types, HCG to syncytiotrophoblastic giant cells and AFP to discrete areas of embryonal carcinoma, particularly the "endodermal sinus tumor" components. These findings provide an explanation for the discordance of serum levels of HCG and AFP frequently observed, and strongly suggest that an elevated serum level of HCG or AFP reflects the presence of the corresponding cell type within the tumor. This information can be used in directing treatment toward the actual tumor cell types present in a particular patient at a particular time.

Proposed Course:

Further tissue specimens will be subjected to the immunoperoxidase assay for HCG and AFP to substantiate the findings reported above. Fresh frozen tissue specimens are being collected and will be assayed both for tissue HCG and AFP concentrations and for specific localization to determine whether routine fixation and embedding alter the reactivity of the tissue antigens.

Project Description:

Part VI. Endoscopy

Part a. Laparoscopy

Objectives:

Staging information can critically influence the choice of definitive primary therapy for patients with a variety of neoplasms. Most notably, the lymphomas, ovarian cancer, and breast cancer are examples. The ability of diagnostic laparoscopy to accurately map out the extent of intra-abdominal spread in several different malignancies with a propensity to metastasize to liver, ovary, and peritoneal surfaces remains an open question. The Surgery Branch

maintains a continuing interest in defining an appropriate role for this endoscopic procedure.

Laparoscopic tubal ligation, as a consultative service, is available for appropriate patients.

Methods Employed:

The diagnostic and therapeutic procedures are performed in the operating room under general anesthesia with Storz equipment.

Major Findings:

During the past year, seven patients have undergone laparoscopy; five for diagnostic reasons and two for tubal ligation.

Recent approval and activation of the Surgery Branch rectal cancer protocol promises to increase our utilization of this endoscopic procedure in the coming year (see colorectal cancer section).

Significance to Cancer Research and the Program of the Institute:

Laparoscopy incurs less patient morbidity, mortality and expense than does laparotomy. By permitting better assessment of tissues only poorly evaluable by present clinical, chemical roentgenographic, and radioisotopic scanning techniques appropriate utilization of this minimally invasive endoscopic procedure should contribute to improving the overall quality of care for certain cancer patients.

Proposed Course:

The sensitivity and accuracy of diagnostic laparoscopy will be determined in the prospective, randomized rectal cancer protocol.

Diagnostic and therapeutic laparoscopy will remain as a Surgery Branch consultative support service.

Project Description:

Part b. Colonoscopy

Objectives:

Fiberoptic endoscopy of the lower GI tract is available as a consultative service function of the Surgery Branch. The utility of this procedure from both the diagnostic and therapeutic point of view of colon lesions is well established.

Methods Employed:

Olympus coloscopes of both the short (110 cm) and long (190 cm) type are available.

Major Findings:

Eleven colonoscopic procedures were performed in the past year. These included seven diagnostic examinations and four snare polypectomies.

Due to departure of the principal investigator in the protocol assessing saline lavage preparation for colonoscopy, this study has been terminated. Available data are inevaluable.

Significance to Cancer Research and the Program of the Institute:

Direct visual access to and ability to histologically sample tissues from the entire lower GI tract offer excellent opportunities for earlier diagnosis of colon malignancy.

Proposed Course:

Colonoscopy will play a role in the preoperative assessment of appropriate patients under consideration for the Surgery Branch rectal cancer protocol.

This minimal morbidity technique will remain available to the Clinical Center patient as a Surgery Branch consultative function.

Project Description:

Part VII. Computer Applications to Research and Clinical Problems in the Surgery Branch

Part a. Serum and Lymphocyte Inventory

Objectives:

The objective of this project is to maintain an inventory of blood component records which are well characterized clinically and are readily accessible.

Methods Employed:

The Serum Inventory System operates with the following characteristics:

(1) The initial inventory records were designed to contain:
    (a) Biographical patient information used to identify the individuals (name, age, sex, etc.).
    (b) Coded descriptions of each patient's primary tumor by site, histology, and extent using the Manual of Tumor Nomenclature and Coding (MOTNAC).

(c) Serum identification number with corresponding patient identification number, date of acquisition, and the quantity of serum vials cryopreserved.
(d) Assay data generated from specific serum samples.

(2) During the past year the system has been expanded to include inventory information on plasma and lymphocyte components of blood drawn from the patients.
(3) Data is entered using the NIH/DCRT WYLBUR system.
(4) The editing and updating programs were generated by the DCRT Data Management System and the information is maintained on magnetic tapes.
(5) Retrieval and display programs were written using the Inquiry and Reporting System (IRS).

Major Findings:

By March 1976, 4517 patient inventory records had been stored in the data base. From 6481 separate blood drawings the following components have been frozen in liquid nitrogen and catalogued in the inventory system:

	Inventory Records	Vials	
Serum	6422	21957	(All plasma samples
Plasma	220	0	are assayed
Lymphocytes	506	2918	immediately.)
Total	7148	24875	

Significance to Cancer Research and the Program of the Institute:

The development of a blood component inventory system is of central importance in longitudinal serologic studies of cancer patients.

Proposed Course:

These studies will continue. The serum and lymphocyte bank will continue to expand.

Project Description:

Part b. Immune Evaluation Visit

Objectives:

The objectives for computer processing information derived from the initial evaluation visit of a patient considered for a randomized protocol are the same as those described in Part c.

Methods Employed:

Initial evaluation visit data collection forms have been designed using the same concepts described in Part c. The information recorded includes WBC,

differential, percentages of T cells and B cells, lymphocyte response to PHA stimulation, and skin test results. It then becomes a part of the Randomized Protocol System data base.

Major Findings:

By the end of March 1976, 483 initial evaluation records had been stored in the system.

A major program was written to select and display information from the Randomized Protocol master file, which includes the initial evaluation records and the Serum Inventory serum query file. The records are sorted by hospital number and date to form a chronological listing of events for each patient. This report is used to then select serum samples for longitudinal immune response studies. (See 3811 - III.)

Significance to Cancer Research and the Program of the Institute:

This information is a vital component of the Randomized Protocol System.

Proposed Course:

Data collection and analysis will continue.

Project Description:

Part c. Randomized Protocols

Objectives:

A data base information management system has been designed and implemented for the randomized protocols recently initiated in the Surgery Branch. The objectives for this system are to:

(1) Store minimal but extremely accurate information on each patient.
(2) Permit the information to be easily keyentered, verified, corrected, retrieved, and analyzed.
(3) Use data collection forms and entry procedures which provide maximum flexibility; i.e., where items may be added or deleted from protocols or field sizes changed as the study progresses.
(4) Use information retrieval techniques which are minimally or easily modified when the data base structure changes.
(5) Produce routine summaries of the information so that it is available and helpful in the ongoing follow-up of each patient.
(6) Make use of existing utility, statistical, and life table analysis programs for the generation of survival rates, recurrence rates, disease-free intervals, and other statistics describing the characteristics of the protocol populations.

Methods Employed:

The Randomized Protocol System incorporates the following methods:

(1) Data collection is performed by a research nurse who is thoroughly familiar with and actively participates in the implementation of the protocols. This helps ensure accuracy and consistency of the information.
(2) The data forms are designed in three parts: Evaluation, Treatment, and Follow-up. Each part contains several sections of data elements to be collected as specified by the protocol. The forms are stored in a WYLBUR data set so they may be easily revised as necessary.
(3) Data is entered using WYLBUR with data entry formats corresponding to sections on the forms.
(4) The data base is updated using IRS and DCRT utility procedures.
(5) All data is 100% verified by visually comparing a listing of the information after it is entered against the source data forms.
(6) Data base entries may be corrected directly using WYLBUR text editing commands.
(7) A record of information in the data base corresponds to a section of information on a data form and is uniquely identifiable for each patient.
(8) IRS is used to produce routine reports as well as to reformat information for the more complicated analytical programs which are generally written in FORTRAN.

Major Findings:

Four randomized protocols have been implemented into the system during the past year. So far most of the data processing has been in the collection and entry of the patient information. The following table gives statistics on information in the data base as of March 1976:

	Protocol Patients		Nonprotocol Patients*	
Disease Protocol	No. of Patients	No. of Records	No. of Patients	No. of Records
SB-M1 Malignant Melanoma	11	98	17	79
SB-S1 Soft Tissue Sarcoma of Extremities	12	151	16	80
SB-S2 Soft Tissue Sarcoma of Head, Neck, Trunk	4	47		
SB-S3 Osteogenic Sarcoma	21	354	5	26
Total	48	650	38	185

*Patients not qualified for the protocol whose disease courses are also being followed.

Significance to Cancer Research and the Program of the Institute:

This system enables the Surgery Branch to maintain up-to-date records of individuals being followed in a readily accessible and useful form. The data accumulated by this project will be used to generate statistics concerning recurrence and survival in the individual diseases that are being treated.

Proposed Course:

As new randomized protocols are developed the system will be extended to include the additional information.

Project Description:

Part d. Nutrition Studies

Objectives:

A data base information management system has been designed and implemented for studies conducted by the Metabolism Section of the Surgery Branch. These include Total Parenteral Nutrition, Nutrition and Head and Neck Cancer, Hyperparathyroid, and Medullary Cancer of the Thyroid. The objectives of the system are the same as those described in Part c.

Methods Employed:

The Nutrition Studies System has been designed using the same methods as those for the Randomized Protocol System. Data collection forms have been designed to meet the specific needs of each study. Additional features of the system include:

(1) A table look-up program written in FORTRAN to identify and produce daily totals of the components of the solutions administered in the Total Parenteral Nutrition study. These totals are then stored in the data base.
(2) Programs which use the clinical and hematology laboratory values stored in the Clinical Information Utility System. These values are combined with nutritional intake values to produce a report of the daily balances of fluids, nitrogen, and electrolytes.
(3) Programs which use the Integrated Plotting Package routines developed by DCRT. They produce graphs of nutritional intake and output parameters over a period of time.

Major Findings:

The following table shows the content of the data base as of March 1976:

Study	Patients	Records
Total Parenteral Nutrition	13	1794
Nutrition and Head and Neck Cancer	0	0*
Hyperparathyroid	0	0*
Medullary Cancer of the Thyroid	0	0*
Total	13	1794

*Forms, formats, and data base structures have been completed and information is currently being collected.

Significance to Cancer Research and the Program of the Institute:

There are many aspects of intravenous feeding, as an adjunct to medical and surgical care, in which exact requirements are not yet known. Accurate recording and storage of experimental data with subsequent analysis will aid the effort to determine the requirements for various nutrients and the efficacy of infusions.

Proposed Course:

Plans are being formulated to implement a cooperative protocol with the Pediatric Oncology Branch for nutritional studies in children. Data collection and analysis will continue on the current studies.

Project Description:

Part VIII. Surgical Treatment of Rectal Cancer

Objectives:

To evaluate in a randomized, prospective fashion the role of electrofulguration alone and in combination with immunotherapy in the definitive treatment of rectal cancer. To assess the sensitivity and accuracy of preoperative laparoscopy in determining those patients who present with Stage D (disseminated) rectal cancer.

Methods Employed:

The Surgery Branch has recently had approved and activated a three-armed study designed to compare electrofulguration alone or electrofulguration plus adjuvant BCG immunotherapy with abdominoperineal resection.

Major Findings:

This program is just beginning and no findings are available as yet. Allowing time for appropriate follow-up, it is anticipated that an accrual of 50 patients to each arm will permit assessment of the relative merit of the enumerated alternatives.

Significance to Cancer Research and the Program of the Institute:

Colorectal cancer is the commonest visceral malignancy in man and kills more patients than any other tumor. Those patients with disease confined to the lower rectum (about one-third of the total) will all undergo abdominoperineal resection. This procedure carries a 5 to 10 percent operative mortality, a 50 percent probability of significant operative morbidity, the prospect of a permanent colostomy with its attendant emotional and psycho-social liability, and still only cures about 40 percent of the patients so treated. By comparison, the several noncontrolled published experiences with electrofulguration report no mortality, significantly less morbidity, no need for colostomy, and survival rates at least comparable to those of abdominoperineal resection.

If it can be shown in a prospective, appropriately controlled study that electrofulguration either alone or with adjuvant immunotherapy is at least as good as or better than abdominoperineal resection, a significant contribution to the management of these patients will have been made.

Proposed Course:

Patient accrual to this clinical program is being actively pursued.

Project Description:

Part IX. The Effects of Thymosin in Patients with Carcinomas of the Lung and Esophagus Treated by Radiation Therapy

Objectives:

To determine if (1) doses of thymosin that have not been toxic thus far in patients with solid malignancies are toxic when given in combination with radiation therapy; (2) to determine if thymosin alters the progressive decline in peripheral blood T cell levels that occurs in patients receiving radiation therapy via mediastinal portals; (3) to determine if thymosin improves the clinical response of patients receiving radiation therapy, and (4) to determine if thymosin affects the disease-free interval and survival when used as an adjuvant to radiation therapy in patients with locally advanced carcinomas of the lung and esophagus.

Methods Employed:

Thymosin fraction V isolated by Dr. Allan Goldstein consists of approximately 12 polypeptides with molecular weights ranging from 1,200 to 14,000. Administration of thymosin to children with thymic deficiency states has resulted in elevation of T cell levels, improvement in cell-mediated responses, and a decrease in the incidence of infections. In patients responding to this treatment, initial T cell levels were low but increased after *in vitro* incubation with thymosin. After several weeks of treatment with thymosin, the peripheral blood T cell levels increased, but no longer further increased after *in vitro* incubation with thymosin. In our study of patients undergoing

irradiation to the mediastinal region for inoperable carcinomas of the lung and esophagus, we found that T cell levels declined during radiation therapy but as a population increased in vitro after incubation with thymosin. By contrast, mean T cell levels did not increase in blood specimens from patients receiving head and neck or pelvic irradiation after in vitro incubation with thymosin. With the hypothesis that mediastinal irradiation may result in a relative deficiency of factors critical for the maturation of thymus-dependent lymphocytes, a randomized prospective study was devised to determine the effects of thymosin administered to patients with carcinomas of the lung and esophagus during radiation therapy. The patients will be stratified into those with (1) epidermoid carcinoma of the lung, (2) adenocarcinoma or large cell carcinoma of the lung, and (3) epidermoid carcinoma of the esophagus. Each group of patients will be randomized to receive either (1) thymosin 20 mg/m^2, (2) thymosin 60 mg/m^2, or (3) placebo. The agents will be administered in a double blind fashion by subcutaneous injection three times a week during radiation therapy. In addition to the usual study parameters in evaluation of treatment regimens for these malignancies, T cell levels before and after in vitro incubation with thymosin will be determined weekly. Each group will be evaluated for response of the tumor as determined on chest x-ray, change in performance status, change in weight, clinical improvement and survival.

Major Findings:

This protocol was recently initiated and is inevaluable at this time, except that none of the patients have developed signs or symptoms attributable to injection of foreign material.

Significance to Cancer Research and the Program of the Institute:

Carcinoma of the lung is the most common malignancy occurring in the United States. Both carcinomas of the lung and carcinoma of the esophagus are usually incurable when diagnosed. Radiation therapy is palliative in sufficient numbers of patients to justify its use, but no systemic agents thus far tested have had a significant palliative effect. If thymosin in combination with radiation therapy in this trial increases the remission rate and interval over that achieved with irradiation alone, it will thus represent a major advance in the treatment of these malignancies.

Proposed Course:

With 24 patients each receiving the two dose regimens of thymosin or the placebo, it is estimated that there will be an 80% probability of detecting a statistically significant effect of thymosin if the expected drop in percent T cells due to radiotherapy is reduced from a mean of 12% to a mean of 4%. This estimate is based on standard formulas, assuming that change in percent T cell levels for a patient is normally distributed with a standard deviation estimated from the in vitro data as approximately 7.0. Therefore, when these numbers have been accrued, the data will be analyzed and correlations made with response to treatment and clinical course.

Project Description:

Part X. The Assessment of Pulmonary Nodules in Patients with Extrathoracic Malignancy

Objectives:

Pulmonary nodules are often the first evidence of recurrence in patients with extrathoracic malignancies. This study investigated the value of whole lung tomograms in determining the extent of metastatic involvement and their impact on survival following resection of pulmonary metastases.

Methods Employed:

Patients with pulmonary nodules and previous or concomitant extrathoracic malignancy who underwent thoracotomy were reviewed. Chest x-ray and pulmonary tomogram findings were correlated and then compared to thoracotomy findings. Survival was then analyzed in patients who underwent resection of all gross metastatic tumor.

Major Findings:

One hundred fifty-two patients have undergone 182 thoracotomies for the evaluation of pulmonary nodules. Chest tomograms demonstrated nodules in 25 patients with normal chest x-rays, showed occult bilaterality in a significant percentage of patients (31% of patients with primary sarcoma), and often revealed more nodules than were present on plain chest x-ray. Survival in the carcinoma group was 50% and in the sarcoma group was 32% at 5 years.

Significance to Cancer Research and the Program of the Institute:

This study has demonstrated the value of routine whole lung tomograms in patients with extrathoracic malignancy to more accurately assess the extent of tumor, demonstrate occult bilaterality, and improve survival in those patients who could have all roentgenographically and thoracotomy-demonstrated tumor resected.

Proposed Course:

Chest tomograms will continue to be utilized to evaluate patients upon presentation to the NCI, for follow-up examinations in selected patients, and to assess the extent of pulmonary involvement in patients with abnormal chest x-rays. Thoracotomy for definitive diagnosis and treatment with postoperative chemotherapy may be compared to this retrospective study to determine the efficacy of future treatments when compared to surgery alone.

Project Description:

Part XI. Nutritional Problems of the Cancer Patient

Objectives:

There is a current major emphasis on central venous hyperalimentation in the cancer patient. Several claims have been made as to the increased efficacy and tolerance to both chemotherapy and radiotherapy following surgery and in some cases claims have been made for the reversal of anergy by nutritional support. The Surgery Branch has a firm commitment to try and elucidate these claims in the cancer patient.

Methods Employed:

The first study which is currently being initiated is a study of the use of adjunctive nutritional support in the management of patients on the Pediatric Oncology Service suffering from rhabdomyosarcoma and neuroblastoma.

Major Findings:

These studies have begun in the past few months.

Significance to Cancer Research and the Program of the Institute:

Evaluation of the nutritional status of cancer patients to develop rational nutritional manipulations may lead to improvements in cancer therapy.

Proposed Course:

In addition to the study with the rhabdomyosarcoma and neuroblastoma patients, the Branch is endeavoring to set up a control randomized trial in the use of adjunctive chemotherapy and Stage III melanoma employing nutritional support in these particular groups. That protocol has yet to be initiated.

Publications:

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Lecky, J.H., Twomey, P.L., Hume, R., and Chretien, P.B.: The effects of N_2O-morphine anesthesia on white cell function in human volunteers. Anesthesdia and Analgesia 54:203-204, 1975.

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Scott, R.N., Faraci, R.P., Goodman, D.G., Militano, T.C., Geelhoed, G.W., and Chretien, P.B.: The role of inflammation in bronchial stump healing. Ann. Surg. 181:381-385, 1975.

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Alexander, J.C.,Jr., Beazley, R.M., and Chretien, P.B.: Mesenteric leaf repair of pelvic defects following exenterative operations. Ann. Surg. 182: 767-769, 1975.

Neifeld, J.P., Doppman, J.L., and Chretien, P.B.: Congenital pelvic arteriovenous fistulas: Report of a case and review of the literature. J. Urol. 114:648-652, 1975

Rosenberg, S.A., and Harken, A.H.: A simple test to determine the site of bleeding in the upper part of the gastrointestinal tract. Surg. Gynec. Obstet. 142:241-242, 1976.

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In Press:

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NOTICE OF INTRAMURAL RESEARCH PROJECT

201 CM 03807-02 SURG

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (50 characters or less)

Immunoevaluation of Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. B. Chretien	Assistant Chief, Surgery Branch	SURG NCI
OTHER:	N. A. Silverman	Clinical Associate	SURG NCI
	J. C. Alexander, Jr.	Clinical Associate	SURG NCI
	C. Potvin	Visiting Scientist	SURG NCI
	D. E. Kenady	Clinical Associate	SURG NCI
	N. H. Goldberg	Clinical Associate	SURG NCI

COOPERATING UNITS (if any)
None

LAB. BRANCH
Surgery Branch

SECTION
Tumor Immunology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

Correlations of peripheral blood thymus-dependent lymphocyte (T cell) levels as determined by the formation of E rosettes with sheep red blood cells and cell-mediated immunity emphasize the need for an objective means of determining T cell levels. A technique for determining T cell levels by cytofluorographic analysis utilizing glutaraldehyde fixation of the cells was devised and demonstrated to correlate with T cell levels as determined by light microscope counting. The in vitro assay of lymphocyte reactivity to PHA and the assay for T cell levels derived in this laboratory were shown to correlate with tumor burden in patients with melanoma: patients with localized or regional disease did not differ from normals; however, those with disseminated disease had mean levels lower than normals. Healthy subjects with a history of cigarette consumption of 20 pack-years or less or those who were less than 40 years of age had increased in vitro lymphocyte reactivity and T cell levels compared to nonsmokers. After cessation of smoking, lymphocyte reactivity returned to normal within 3 months; however, T cell levels remained elevated compared to normal controls. These observations define another important variable that must be considered in the interpretation of these in vitro correlates of cell-mediated immunity.

PHS-6040

Project Description:

Part I: Determination of Peripheral Blood E Rosette Levels by Cytofluorographic Analysis

Part II: In Vitro Lymphocyte Reactivity and T Cell Levels in Patients with Melanoma: Correlations with Clinical and Pathologic Stage

Part III: In Vitro Lymphocyte Reactivity and T Cell Levels After Cessation of Chronic Cigarette Smoking

Project Description:

Part I: Determination of Peripheral Blood E Rosette Levels by Cytofluorographic Analysis

Objectives:

The formation of E rosettes (ER) by human peripheral blood lymphocytes after incubation with sheep red blood cells is thought to be a function of thymus-dependent lymphocytes (T cells). T cell levels correlate with cell-mediated immunity in patients with solid malignancies, and patients with thymus-dependent immune deficiency diseases have abnormally low T cell levels. These and other studies suggest a potential usefulness for a determination of peripheral blood T cell levels as an index of cell-mediated immunity. Conventionally, T cell levels are determined by light microscope counting (LMC) of the percentage of lymphocytes with a rosette of sheep red blood cells in a wet preparation placed on a hemocytometer chamber. This technique is tedious, subjective, and leads to significant operator fatigue. These factors limit the number of samples that can be processed each day. The purpose of this investigation was to develop a rapid, objective method of determining T cell levels utilizing the Cytofluorograf, a rapid flow-through cytofluorometer.

Methods Employed:

Lymphocytes were separated from peripheral blood by Ficoll-Hypaque gradient. The cell layer was pipetted off, washed in RPMI 1640 culture medium, and adjusted to a final concentration of approximately $2x10^6$ cells/ml. To a .25 ml aliquot of this cell suspension .25 ml of 1% sheep erythrocytes and .1 ml of AB serum was added and the mixture incubated for 30 minutes at 4°C. This preparation was incubated for 15 minutes at 37°C, centrifuged at 200g for 5 minutes, refrigerated at 4°C for approximately 18 hours, and then the percent ER's determined by LMC and cytofluorographic analysis (CFGA). Smears made at this time of samples processed in an identical fashion, except for the addition of sheep erythrocytes, contained 97-100% lymphocytes. To the cell pellet utilized for CFGA .2 ml of 1% glutaraldehyde was added and the mixture incubated at 4°C for at least 20 minutes. 4 ml of acridine orange dye in a concentration of 2 µg/ml in phosphate-buffered saline were added

to the sample, and 4-8 minutes later the percent ER's were determined by CFGA. As the lymphocyte distribution appeared on the cathode ray tube, a window limited to rosetted lymphocytes was selected using the radius, slope, and balance controls. After selecting the window, 10,000 cells were counted and the percent of ER's determined from the selected count display.

Major Findings:

Determination of ER levels from 8 samples determined by LMC with and without glutaraldehyde and by CFGA with glutaraldehyde showed that glutaraldehyde increased the percent ER levels determined by LMC, however, ER levels determined by LMC without glutaraldehyde and CFGA with glutaraldehyde did not differ significantly. Single determinations of ER levels counted by CFGA and by LMC were compared. There was good correlation between these techniques. To compare reproducibility, duplicate samples were counted. Again there was good correlation between CFGA and LMC when the average values were compared. There was, however, relatively less variation between each group of duplicate samples analyzed by CFGA. A final comparison was made between single countings by CFGA and LMC using triplicate counting and discarding any determination that deviated more than 10% from the mean of the 3 counts. There was excellent correlation between CFGA and LMC in this comparison. The counting of 12 samples by CFGA required 30 minutes, while counting these samples in triplicate by LMC required 2 hours.

Significance to Cancer Research and the Program of the Institute:

The correlations between *in vitro* assays that parallel *in vivo* cell-mediated immunity and tumor burden, response to treatment, and clinical course in cancer patients give rise to a need for a simple reproducible and objective assay that correlates with cell-mediated immunity. Accumulating evidence that peripheral blood E rosette levels provide an index of cell-mediated immunity give importance to the development of a technique for the performance of the assay by a more objective method than light microscope counting. The technique developed in this study appears to fulfill these needs.

Proposed Course:

Preliminary investigations with lymphocytes from patients receiving radiation therapy and chemotherapy indicate that the CFGA technique does not correlate as well with LMC as in untreated patients and normals. This discrepancy can be accounted for by partial damage to the lymphocytes by the therapies, resulting in a variation in the incorporation of acridine orange. Theoretically, this difficulty can be overcome by utilizing a three parameter analysis as opposed to two parameter analysis utilized in this experiment. Instrumentation capable of such analysis has been ordered, and upon receipt, will be utilized to develop a method for determination of ER levels by cytofluorographic analysis in patients receiving radiation therapy and chemotherapy.

Project Description:

Part II. In Vitro Lymphocyte Reactivity and T Cell Levels in Patients with Melanoma: Correlations with Clinical and Pathologic Stage

Objectives:

Studies of the effects of immunotherapy in patients with melanoma show a correlation between tumor burden and response to this treatment. Reduction of tumor burden by operation or systemic chemotherapy appears to be an important antecedent for obtaining a beneficial effect with BCG immunotherapy. Implicit in the selection of patients for such cytoreductive therapy is the accurate determination of the extent of tumor present. In patients with melanoma, clinical examination and diagnostic evaluations often fail to detect the presence of regional and distant metastases. This study was undertaken to determine the contribution of *in vitro* reactivity of peripheral blood lymphocytes and peripheral blood T cell levels to the assessment of tumor burden.

Methods Employed:

Forty-two patients with melanoma were studied. None had had previous treatment. They were classified as clinical Stage I (localized disease), Stage II (regional metastases), or Stage III (disseminated metastases). After completion of an extensive clinical and laboratory evaluation, patients with clinical Stage I and II disease had operative removal of all demonstrable tumor, and patients with Stage III disease had biopsy of the disseminated lesions. They were then classified on the basis of the histologic examination of the operative specimens by pathologic stage (Stage I, regional lymph nodes histologically negative for metastases; Stage II, regional lymph nodes histologically positive for metastases; and Stage III, histologic documentation of distant metastases).

Prior to initiation of the preoperative evaluation, peripheral blood *in vitro* lymphocyte reactivity and T cell levels were determined in all patients. Peripheral blood lymphocytes were separated on a Ficoll-Hypaque gradient, washed in RPMI culture media, resuspended, and counted. Viability was determined by Trypan blue exclusion. For determination of lymphocyte reactivity to PHA, culture tubes in triplicate containing 150,000 lymphocytes in .2 ml of 90% RPMI 1640 and 10% AB serum were incubated for 72 hours after the addition of four concentrations of PHA. Tritiated thymidine was added 6 hours prior to determination of the culture. The cells were harvested by the Multiple Automated Sample Harvester onto filter strips, the strips dried and placed into scintillation counting vials containing toluene-Liquiflor. Incorporation of tritiated thymidine was quantitated by scintillation counting and the absolute activity expressed in disintegrations per minute. Lymphocyte reactivity was defined as the mean of the triplicate samples with the highest disintegrations per minute minus the disintegrations per minute of the control cultures. For a determination of T cell levels,

$2x10^6$ mononuclear cells/ml samples were prepared and .25 ml of this suspension was added to .2 ml of sheep erythrocytes in RPMI 1640 and .1 ml of AB serum. These preparations were incubated at 37°C for 15 minutes, centrifuged and allowed to stand for approximately 18 hours at 4°C prior to counting. Aliquots of this suspension were transferred to a hemocytometer and the percentage of cells forming rosettes were calculated after counting 200 lymphocytes. All samples were done in triplicate.

Major Findings:

The mean lymphocyte reactivity of all melanoma patients did not differ significantly from age-matched normal controls. In addition, the reactivity of patients with clinical Stage I did not differ from controls, but the reactivity of patients with clinical Stage II tumors was significantly lower than controls ($p < .02$) and patients with Stage II tumors ($p < .05$). When the patients were reclassified by pathological stage based on the finding of solitary metastases on preoperative evaluation in 6 patients, the mean lymphocyte reactivity of patients with pathological Stage I and II tumors did not differ from that of patients with clinical Stage I tumors, the controls, or from each other. However, the mean lymphocyte reactivity of patients with pathological Stage III tumors was significantly lower than those with pathological Stage II tumors ($p < .01$). This analysis shows that the impaired lymphocyte reactivity in patients with clinical Stage II tumors was due to the low lymphocyte reactivity of patients with occult distant metastases. The findings with assay of peripheral blood percent T cell levels was similar. The T cell levels of patients with clinical Stage II tumors were lower than those with Stage I tumors but when analyzed by pathological stage, the T cell levels of patients with pathological Stage I and II tumors did not differ from controls, but the mean T cell levels of the patients with pathological Stage III tumors was significantly less than that of the other groups. The lymphocyte reactivity and T cell levels did not correlate with pathological stage in individual patients. Among patients with pathological Stage II tumors, 9 of 19 had lymphocyte reactivity and 7 of 19 had T cell levels within the range of patients with pathological Stage III tumors.

Significance to Cancer Research and the Program of the Institute:

The previous demonstrations of a correlation between tumor burden and response to both conventional and experimental therapy such as immunotherapy give importance to the development of techniques for determination of tumor extent before, during, and after treatment. This study shows that lymphocyte reactivity to PHA and peripheral blood T cell levels correlate with tumor burden in patient groups. Although the assay does not qualify as a critical tumor marker in individual patients, the correlations made serve as a basis for the development of such markers.

Proposed Course:

Future studies will determine the relationship between T cell subpopulations and tumor extent in patients with solid malignancies in an attempt to improve the correlations demonstrated in this study.

Project Description:

Part III. In Vitro Lymphocyte Reactivity and T Cell Levels After Cessation of Chronic Cigarette Smoking

Objectives:

In a previous study we found that in vitro lymphocyte reactivity and peripheral blood thymus-dependent (T cell) lymphocyte levels were increased in chronic smokers less than 40 years of age or with a cigarette consumption of 20 pack-years or less when compared to age-matched nonsmokers. These parameters in older smokers or those with a history of heavier cigarette consumption did not differ from age-matched nonsmoking controls. In this study we assessed the effects of cessation of chronic cigarette consumption on these parameters.

Methods Employed:

Seventy-two persons who consumed cigarettes on a daily basis were studied prior to cessation of cigarette consumption. All were studied at one month after cessation of cigarette consumption, and 52 who abstained from smoking for 3 months were studied also at that interval. Controls were 115 age- and sex-matched normals who never smoked cigarettes and were in otherwise good health. Lymphocyte reactivity to PHA and peripheral blood T cell levels were determined as in Part I.

Major Findings:

The mean lymphocyte reactivity to PHA was higher in smokers than nonsmokers ($p < .01$). This difference was due to an increased lymphocyte reactivity of those with a history of cigarette consumption of 20 pack-years or less. Compared to the lymphocyte reactivity while smoking, the lymphocyte reactivity of all former smokers was significantly lower at one month and at 3 months after cessation of smoking and did not differ from nonsmokers. This decline in lymphocyte reactivity of former smokers was due to the decline in the reactivity in the group whose prior cigarette consumption was 20 pack-years or less. The mean lymphocyte reactivity of those with heavier cigarette consumption was similar to nonsmokers and was unchanged after cessation of smoking. The peripheral blood T cell levels were statistically higher in smokers than nonsmokers ($p < .05$) and remained elevated at one and 3 months after cessation of smoking, regardless of prior smoking history.

Significance to Cancer Research and the Program of the Institute:

The data show that the increased lymphocyte reactivity to PHA in chronic cigarette smokers whose consumption is 20 pack-years or less is reversible. These findings give importance to the correlations between cigarette consumption and risk for developing malignancies of the head and neck region and respiratory tract. The data should serve as the basis for

epidemiological studies that correlate the risk of developing these malignancies with the extent of cigarette consumption and duration and reversibility of this risk after cessation of chronic cigarette consumption.

Proposed Course:

In view of the reversibility of the elevated lymphocyte reactivity to PHA after cessation of chronic cigarette consumption in a group whose peripheral blood T cell levels remained elevated, future studies will explore this dichotomy in an attempt to define and characterize the T cell subpopulations accounting for these findings.

Publications:

Chretien, P.B., Catalona, W.J., Twomey, P.L., and Sample, W.F.: Correlation of immune reactivity and clinical status in cancer. Ann. Clin. Lab. Science 4:331-338, 1974.

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Chretien, P.B., Twomey, P.L., Catalona, W.J., and Hansing, R.L.: Immunologic defects associated with squamous carcinoma. In Anderson, R., and Hoopes, J.E. (Eds.): Symposium on Malignancies of the Head and Neck. St. Louis, C.V. Mosby, 1975, Vol. 11, pp. 13-18.

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Silverman, N.A., Potvin, C., Alexander, J.C.,Jr., and Chretien, P.B.: In vitro lymphocyte reactivity and T-cell levels in chronic cigarette smokers. Clin. Exp. Immunol. 22:285-292, 1975.

In Press:

Tarpley, J.L., Catalona, W.J., and Chretien, P.B.: Effect of previous irradiation on DNCB reactivity in cancer patients. Surgery.

NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 CM 06809-02 SURG

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
In Vitro Responsiveness to Thymosin of Lymphocytes from Patients with Solid Malignancies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATOR AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. B. Chretien	Assistant Chief, Surgery Branch	SURG NCI
OTHER:	C. Potvin	Visiting Scientist	SURG NCI
	D. E. Kenady	Clinical Associate	SURG NCI
	R. M. Simon	Head, Biostatistics and Data Management Section	DCT NCI

COOPERATING UNITS (if any)

Biostatistics and Data Management Section, DCT, NCI

LAB/BRANCH

Surgery Branch

SECTION

Tumor Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	2.0

ABSTRACT OF WORK (200 words or less - underline keywords)

The demonstrations that thymosin, an extract of fetal calf thymus, improves cellular immune responses in children with thymic deficiencies form the basis for a determination of the effects of thymosin in vitro on T cell levels in patients with solid malignancies. In children with low T cell levels, a rise in T cell levels occurred after incubation with thymosin; and after in vivo treatment with thymosin, their T cell levels rose and they improved clinically. Application of this assay to (1) tumor-bearing patients with solid malignancies, (2) patients receiving radiation therapy to head and neck, mediastinal, and pelvic portals, (3) patients clinically cured of solid malignancies, and (4) normal controls all show an inverse relationship between initial T cell levels and the in vitro response to thymosin: With progressively lower initial T cell levels the increase after incubation with thymosin increased progressively. As a group, the greatest increase occurred in patients receiving radiation therapy for mediastinal malignancies. The data constitute a basis for a determination of the effects of thymosin on cell-mediated responses and clinical course in patients with solid malignancies. The findings in patients receiving radiation therapy for mediastinal malignancies suggest that this group may constitute an ideal model for a determination of these effects of the agent.

Project Description:

Part I: In Vitro Effect of Thymosin on T Cell Levels During Radiation Therapy: Correlations with Radiation Portal and Dose, Tumor Histology, and Initial T Cell Levels

Part II: In Vitro Effect of Thymosin on T Cell Levels in Tumor-Bearing and Clinically Cured Cancer Patients

Project Description:

Part I: In Vitro Effect of Thymosin on T Cell Levels During Radiation Therapy: Correlations with Radiation Portal and Dose, Tumor Histology, and Initial T Cell Levels

Objectives:

Recently, thymosin, an extract of fetal calf thymus, improved cellular immune responses in children with thymic deficiencies. The patients had low thymus-derived lymphocyte (T cell) levels which increased after incubation in vitro with thymosin. After treatment with thymosin, their circulating blood T cell levels increased and did not increase further after incubation with thymosin. These correlations suggest that if cancer patients have impaired cellular immunity that is secondary to deficiencies of factors vital for maturation of T cell precursors, thymosin may be similarly effective in increasing T cell levels and other parameters of cellular immunity.

If the thymus gland is the principal source of factors that lead to maturation of T cell precursors, irradiation of the mediastinal region may cause a decline in cellular immune response due to deleterious effects of irradiation on this function of the gland. If this mechanism is a major contributor to the decline in cellular immunity during mediastinal irradiation, then T cell levels in blood specimens from patients during mediastinal irradiation may increase after incubation with thymosin as occurred in children with thymic deficiencies.

The objective of this study was to explore these hypotheses by determining the comparative effects of thymosin in vitro on T cell levels in cancer patients receiving radiotherapy via mediastinal, head and neck, and pelvic portals.

Methods Employed:

The effect of thymosin in vitro on percent T cells was determined in 388 blood specimens from patients with head and neck, mediastinal, and pelvic malignancies during radiation therapy, in 94 untreated patients with these malignancies, and 277 normal adults. All patients had clinically localized tumors that were encompassed by the radiation portal. Peripheral blood T cell levels were quantitated by the determination of the percent of lymphocytes forming spontaneous rosettes when mixed with sheep erythrocytes.

Triplicate samples were prepared for T cell determinations and 3 additional samples were prepared in an identical fashion with the addition to each tube of .1 ml of phosphate-buffered saline containing 100 µg of thymosin fraction V provided by Dr. Allan Goldstein. The tubes were coated so as to prevent identification of the blood donor or of the tubes containing thymosin by the person performing the T cell determinations. The effect of thymosin was determined by subtracting the mean of the triplicate samples without thymosin from the mean of the triplicate samples with thymosin.

Major Findings:

After incubation with thymosin, the mean T cell level in normals increased significantly ($p < .005$). In untreated patients with head and neck and pelvic malignancies the levels also increased significantly, but did not differ from the increase in normals. In untreated patients with mediastinal malignancies, however, the increase was not significant. Mean T cell levels in patients with head and neck and pelvic malignancies during irradiation did not change significantly after incubation with thymosin, and in pelvic malignancies the change was less than untreated patients ($p < .02$). However, in patients with mediastinal malignancies during irradiation the mean level increased ($p < .001$) and the increase was greater than in normals ($p < .05$). An additional analysis was done to ascertain whether the effect of thymosin correlated with tumor histology in patients receiving radiation therapy. Since all head and neck malignancies were squamous carcinomas, the mediastinal and pelvic malignancies were subdivided into squamous and nonsquamous carcinomas. There was no significant difference in the change in T cell levels after incubation with thymosin between those with squamous and those with nonsquamous carcinomas. This analysis shows that the significantly increased mean T cell level in patients with mediastinal malignancies did not correlate with tumor histology. Determinations of the relation between cumulative radiation dose and change in T cell levels showed no correlation in any of the groups studied. On the other hand, regression analysis showed a correlation between initial percent T cell levels and the effects of thymosin in all groups studied. There was a significant inverse relation between initial T cell levels and T cell levels after incubation with thymosin.

Significance to Cancer Research and the Program of the Institute:

The major finding in this study is that thymosin in vitro increases the percent T cell levels in both normals and cancer patients and the effect varies inversely with the initial T cell level. Furthermore, in patients receiving radiation therapy the increase in patients with mediastinal malignancies for a given T cell level was greater than in patients with pelvic malignancies, and as a group was greater than in patients with head and neck malignancies. If the data from the treatment of children with thymic deficiency apply, the data suggest that thymosin may increase T cell levels in patients with low T cell levels as a population. Thus, thymosin may be a useful immunoadjuvant in patients with low T cell levels associated with tumor burden or secondary to effects of immune suppressive therapy. The singular effects on T cell levels in patients with mediastinal malignancies during radiation

therapy form a rationale for the determination of the effects of thymosin in combination with radiation therapy in patients with mediastinal malignancies.

Proposed Course:

The findings in this study constitute the basis for a prospective randomized study to determine the effects of thymosin in combination with radiation therapy in patients with carcinomas of the lung and esophagus localized to the thorax. Additional in vitro studies will be conducted that will further characterize the lymphocyte subpopulation accounting for the differences seen with the effects of thymosin in these patients.

Project Description:

Part II. In Vitro Effect of Thymosin on T Cell Levels in Tumor-Bearing and Clinically Cured Cancer Patients

Objectives:

The demonstrations that children with thymic deficiencies improve clinically after treatment with thymosin and manifest increase in T cell levels and other parameters of cell-mediated immunity suggest a possible application of this agent in the treatment of patients with solid malignancies and suppressed cell-mediated immunity. In the children with thymic deficiencies, response to thymosin treatment could be predicted by the in vitro response of lymphocytes to thymosin: A rise in T cell levels as determined by rosette formation with sheep red blood cells was predictive of a response to thymosin in vivo. This correlation formed the basis for a screen of tumor-bearing patients with solid malignancies and patients clinically cured of these tumors.

Methods Employed:

The effect of thymosin in vitro on percent T cells was determined in over 200 patients with clinically localized malignancies, 127 patients with no evidence of recurrence 5 or more years after tumor resection, and in 277 normals. The tumors represented were squamous carcinomas of the head and neck region, lung, esophagus, and cervix; adenocarcinomas of the lung, breast, and rectum; melanomas; and sarcomas. Patients with evidence of vital organ dysfunction or a history of previous malignancy were excluded. Peripheral blood T cell levels were quantitated by determination of the percent of lymphocytes forming spontaneous rosettes when mixed with sheep erythrocytes. Identical samples were also prepared with the addition of 100 µg of thymosin fraction V provided by Dr. Allan Goldstein. All samples were performed in triplicate and were coded and randomized so as to prevent identification of the donor by the person performing the T cell determinations. The effect of thymosin was defined as the mean of triplicate samples without thymosin minus the mean of triplicate samples incubated with thymosin.

Major Findings:

In normals, tumor-bearing patients, and patients clinically cured of malignant tumors by surgery, T cell levels after incubation with thymosin showed an inverse relationship with initial T cell levels. With progressively lower T cell levels, there was a progressively greater increase in the T cell levels after incubation with thymosin as determined by regression analysis. There were no remarkable correlations with tumor burden or histology. The slopes of the regressions derived from the effect of thymosin on tumor-bearing and clinically cured patients were identical and both were less than the slope of the regression derived from normals.

Significance to Cancer Research and the Program of the Institute:

The study shows that the in vitro effect of thymosin on T cell levels is related to the initial T cell level. This relationship suggests a potential value of the agent in elevating T cell levels in cancer patients with low T cell levels.

Proposed Course:

The findings of this study provide a rational basis for the determination of the effects of thymosin on cell-mediated immunity in patients with suppressed responses. Additional investigations will be required to determine whether this effect of thymosin occurs during general anesthesia and surgery and during the administration of immunosuppressive chemotherapy. If so, trials of the beneficial effect on cellular immune responses and course after cancer therapy of thymosin would appear warranted.

Publications:

In Press:

Kenady, D.E., Potvin, C., Simon, R.M., and Chretien, P.B.: Effect of thymosin in vitro on T cell levels in cancer patients. Cancer.

Kenady, D.E., Simon, R.M., and Chretien, P.B.: Effect of thymosin in vitro on T cell levels during radiation therapy: Correlations with radiation portal and dose, tumor histology, and initial T cell levels. Amer. J. Roentgenol.

NOTICE OF
INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

July 1, 1975 to June 30, 1975

TITLE OF PROJECT (80 characters or less)

Studies on the Circulating Glycoproteins of Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PARTICIPATING SCIENTISTS (INDICATE PROFESSIONAL PERSONNEL CHARGED TO THE PROJECT

PI:	P. B. Chretien	Assistant Chief, Surgery Branch	SURG NCI
OTHER:	N. A. Silverman	Clinical Associate	SURG NCI
	J. C. Alexander, Jr.	Clinical Associate	SURG NCI
	W. P. Bradley	Research Chemist	AFRRI
	A. S. Evans	Research Chemist	AFRRI
	J. F. Weiss	Research Chemist	AFRRI

COOPERATING UNIT (IF ANY)

Armed Forces Radiobiology Research Institute

LAB/BRANCH

Surgery Branch

SECTION

Tumor Immunology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	2.5	2.5

ABSTRACT (250 words or less - underline keywords)

Determination of serum carcinoembryonic antigen (CEA) levels in healthy nonsmokers and smokers shows a progressive increase with age in each group and a consistently higher level in smokers. The results show that the currently used upper limit of normal for this assay is applicable only to nonsmokers of approximately 40 years of age. These findings may increase the utility of the assay as a marker for tumor presence in malignancies associated with elevations of CEA levels. CEA levels in head and neck cancer are elevated only in the presence of clinically manifest disease, thus diminishing the value of this assay as a tumor marker in such patients. Serum carbohydrates and serum glycoproteins show correlations with tumor burden in a variety of solid malignancies. In addition, certain carbohydrates and glycoproteins show an inverse relationship with in vitro lymphocyte reactivity. The studies indicate that additional investigations are warranted to determine the value of these moieties as a marker of tumor burden and correlate of immune reactivity in cancer patients.

FHS-1040

Project Description:

Part I: Effect of Age and Cigarette Smoking on Carcinoembryonic Antigen Levels

Part II: Carcinoembryonic Antigen Levels in Head and Neck Cancer

Part III: Serum Glycoprotein Carbohydrate Levels in Patients with Solid Malignancies

Project Description:

Part I: Effect of Age and Cigarette Smoking on Carcinoembryonic Antigen Levels

Objectives:

After elevated levels of carcinoembryonic antigen (CEA) were found in patients with carcinoma of the colon, it was speculated that CEA was a tumor-specific antigen that would be of value for the identification of patients with carcinoma of the colon and a means of detecting tumor recurrence after treatment. Subsequently, elevated CEA levels were found in patients with carcinomas of the pancreas, lung, stomach, breast, and head and neck region. Furthermore, elevated CEA levels were found in patients with benign diseases such as chronic bronchitis, emphysema, ulcerative colitis, regional enteritis, and alcoholic cirrhosis. Such elevations of CEA levels in nonmalignant diseases limits the usefulness of the antigen as a marker of tumor presence and for monitoring of tumor response to treatment. The most common factor shown to be associated with elevations of CEA levels is cigarette smoking. Previous studies have shown a 15% incidence of elevated CEA levels in normals with a smoking history greater than 15 cigarettes a day compared to only 1.8% incidence in age-matched nonsmokers. The high prevalence of cigarette smoking makes it the most important known factor that may diminish the value of CEA as a tumor marker. The object of this study was to quantitate the effect on CEA levels of cigarette smoking in healthy smokers and determine the effect on CEA levels of cessation of cigarette smoking.

Methods Employed:

Healthy volunteers who were currently smoking cigarettes were studied. Those known to have active acute or chronic diseases and those with a history of malignancy were excluded. Healthy nonsmokers were also solicited and similarly screened. Specimens were obtained prior to cessation of smoking in those chronic smokers who ceased smoking and also obtained at 1, 3, and 6 months from those who maintained abstinence from smoking. CEA assay was performed by the Hansen Z gel technique.

Major Findings:

The mean CEA levels of nonsmokers was 1.9 ng/ml. The upper limit of "normal" for CEA levels was fixed at 2 standard deviations above the mean of nonsmokers

(4.94 ng/ml). CEA levels were determined to the nearest .1 ng, thus levels of 5.0 ng/ml or greater were considered elevated. Six of 122 (5%) nonsmokers had elevated CEA levels, a distribution identical to determination of CEA levels on plasma in nonsmokers, although both the mean and variability of serum CEA levels were greater than plasma CEA levels determined on the same blood tested both as serum and plasma. The mean CEA level of 154 smokers was 2.7 ng/ml and 18 (12%) had elevated levels. The mean CEA level of smokers was .8 ng/ml higher than that of nonsmokers ($p < .001$) and the number of smokers with abnormal CEA levels was greater ($p < .05$). In both smokers and nonsmokers, CEA levels decreased with age. Among nonsmokers the median CEA level rose from 1.4 ng/ml in the third decade to 1.9 ng/ml in those greater than 50 years of age. Among smokers, the median CEA level rose from .9 ng/ml in the third decade to 2.9 ng/ml in those over 50 years of age. Regression of CEA levels on age in both smokers and nonsmokers showed a significant correlation ($p < .01$). CEA levels appeared equivalently related to age in smokers and nonsmokers. Regression of CEA levels on pack-years of cigarette consumption showed a significant correlation ($p < .02$). Age and pack-years of smoking history also correlated ($p < .001$). Seventy-six of the 154 smokers ceased smoking for at least one month and were restudied at 1, 3, and 6 months afterwards, or until they resumed smoking. One month after cessation of smoking, their CEA level was lower than while smoking, but was higher than nonsmokers. Of those studied 3 months after cessation of smoking, their CEA level did not differ from nonsmokers. Thus, all elevated CEA levels in smokers declined to within the range of nonsmokers within 3 months among those who ceased smoking for this interval of time.

Significance to Cancer Research and the Program of the Institute:

The data indicate that smoking has a predictable influence on CEA levels and elevates serum CEA levels approximately .8 ng/ml, regardless of age or amount of cigarettes consumed. Also, after cessation of smoking, CEA levels declined to levels similar to nonsmokers within a 3 month period. This observation is important for the clinical evaluation of CEA levels in former smokers. CEA levels obtained greater than 3 months after the cessation of smoking appear to be evaluable independent of prior smoking history. The correlations of CEA levels with age in both smokers and nonsmokers indicate that a reappraisal of the diseases associated with elevated CEA levels using an upper limit of normal that considers the influence of age and smoking habits may invalidate some of the positive correlations previously reported. Also, consideration of age and smoking history may improve the correlations between CEA levels and tumor burden and thus extend the clinical utility of CEA as a marker of tumor presence.

Proposed Course:

Studies now in progress compare CEA levels with serum levels of glycoproteins to determine whether serum levels of several glycoproteins evaluated as a group may serve as a better index of tumor burden than those glycoproteins elevated in cancer patients when considered singly. An integral part of this analysis is the correlation of CEA levels and the levels of other

glycoproteins being studied with immune reactivity and in vitro correlates of cellular immunity in the patients studied.

Project Description:

Part II. Carcinoembryonic Antigen Levels in Head and Neck Cancer

Objectives:

In previous studies on patients with carcinomas of the lung and head and neck region, elevations of CEA levels have been reported. However, because of the epidemiological association with cigarette consumption and these tumors, the data may not reflect tumor burden but rather may be due to the effects of cigarette consumption. This hypothesis derives from our findings of significant elevations in CEA levels associated with chronic cigarette consumption (Part I). Because of the need for serum markers of tumor burden in monitoring host response to tumor treatment and earlier detection of subclinical residual disease, this study was conducted to correlate CEA levels with tumor burden in patients with head and neck carcinomas using as controls normal subjects of similar age and with similar levels of cigarette consumption.

Methods Employed:

Over 400 patients with squamous carcinomas of the head and neck region were studied. One hundred seventy-six were tumor-bearing at the time of testing, and of these, 31 were studied sequentially at varying intervals from 2 months to 4 years after surgical resection. An additional 23 patients were studied at intervals during definitive radiation therapy. A final group of 147 patients who were tumor-free for greater than 3 years after treatment were studied. The CEA levels were determined on serum samples using the Hansen Z gel assay. The controls were 276 healthy volunteers selected by age and cigarette consumption to match the tumor patients studied.

Major Findings:

The mean serum CEA levels in the smokers was 2.8 ng/ml and the upper limit of normal was set at 2 standard deviations above the mean. This defined the upper limit of normal for nonsmokers was 5.0 ng/ml and for smokers it was 7.0 ng/ml. Five percent of nonsmokers and 13% of smokers had CEA levels that exceeded these limits. Twenty-six (36%) of tumor-bearing patients had CEA levels of 5.0 ng/ml or greater. All gave a history of heavy cigarette consumption. Using the upper discriminate level of 7.0 ng/ml, 30 (17%) had elevated CEA levels, which exceeded the number of elevations in smokers and nonsmokers ($p < .001$). In addition, the number of markedly elevated CEA levels (> 15 ng/ml) was greater in the cancer patients than in smokers or nonsmokers. The levels in the tumor-bearing patients also correlated with tumor burden as determined by clinical staging, with the highest levels occurring in the patients with the most advanced stage of tumor. In patients treated by definitive surgery, there was no correlation between initial CEA level and subsequent clinical course. In 10 patients with elevated

preoperative levels, the levels were below the normal range at 1 month post-operatively. However, among 14 patients who developed recurrence after operation, in 10 the levels were normal at the time of recurrence. In patients with elevated CEA levels who received radiation therapy, the levels declined progressively during treatment, paralleling tumor regression. In patients with normal levels prior to irradiation, the levels did not rise during radiation therapy. Selectively fewer abnormal CEA levels occurred in patients tumor-free less than 2 years than in patients tumor-free greater than 2 years, and in both groups the levels were less than in the tumor-bearing patients. In both groups, the levels were similar to that of the smokers, but were higher than in nonsmokers.

Significance to Cancer Research and the Program of the Institute:

The ideal tumor marker would provide an index of prognosis by indicating the presence of occult metastases in patients with clinically localized tumors. Such correlations have been demonstrated in patients with carcinomas of the colon, lung, and breast. However, the data indicate that CEA is not useful for this purpose in patients with carcinomas of the head and neck region. Relevant to this consideration is the ease with which recurrences can be detected in patients with carcinomas of the head and neck region compared to the relative difficulty in detecting early residual disease in malignancies involving the visceral organs. However, the fall in CEA levels after surgical resection, the lack of elevation in CEA levels during radiation therapy, and the decline in CEA levels as the tumors responded to radiotherapy all indicate that CEA may be useful when combined with other serum markers of tumor presence in determining the response of tumor to treatment and in the earlier detection of metastases.

Proposed Course:

Studies in progress evaluate serum carbohydrate and glycoprotein levels in these patients. Comparisons will be made with CEA and in vitro correlates of immune reactivity in these patients and in patients with other malignancies in an attempt to derive a group of serum markers which correlate with tumor burden and show alterations prior to the development of clinically manifest residual disease. This study demonstrates that the panel of markers derived will vary with tumor histology and anatomical location.

Project Description:

Part III. Serum Glycoprotein Carbohydrate Levels in Patients with Solid Malignancies

Objectives:

Changes in serum glycoproteins have been documented in many pathologic processes including malignancies. Alteration in the serum concentrations of glycoproteins thus may be of potential value in the determination of tumor burden. The carbohydrate moieties of the glycoproteins, neutral hexose,

fucose, sialic acids, hexosamines, also have been shown to be elevated in patients with malignancies. It is relevant here that carcinoembryonic antigen (CEA) is a glycoprotein rich in these carbohydrates and has been shown to parallel tumor burden after treatment in patients with elevated levels of CEA prior to therapy. Additional studies indicate that glycoproteins may suppress immune reactivity. The purpose of this study was to correlate glycoprotein-associated monosaccharides and a group of 17 glycoproteins with tumor burden, lymphocyte reactivity in vitro, and serum CEA levels.

Methods Employed:

The patients studied had a variety of solid malignancies clinically staged as localized, metastatic to regional lymph nodes, and disseminated. All blood specimens were obtained prior to treatment. Controls consisted of healthy adults who did not smoke or drink and an additional group of heavy smokers. The control groups were matched for mean age and for sex with the cancer patients. Serum protein-bound carbohydrates and serum glycoprotein levels were determined in the laboratories of the Armed Forces Radiobiology Research Institute under a contractual arrangement. Serum CEA levels were determined by the Hansen Z gel assay. Lymphocyte reactivity to phytohemagglutinin (PHA) was determined in microculture.

Major Findings:

The mean levels of the protein-bound carbohydrates in tumor-bearing patients were significantly higher than in both nonsmoking and smoking normal controls. When the patients were divided into those with local, regional, and disseminated tumors, the mean levels of each carbohydrate increased progressively with increasing tumor burden. Furthermore, the mean levels between each clinical tumor stage also differed. In a group of patients tumor-free for more than 3 years, the serum-bound carbohydrate levels were lower than that of patients with localized tumors but higher than the controls. The mean serum CEA levels in all subpopulations of patients studied did not differ from normals, was elevated compared to nonsmokers, but did not differ from that of smokers. There was no correlation between serum protein-bound carbohydrate levels and CEA levels. The most consistently elevated glycoproteins were the alpha-1-acid glycoprotein, alpha-1 antitrypsin, haptoglobin, and ceruloplasmin. These proteins showed a relationship to tumor burden similar to the protein-bound carbohydrates. There was an inverse relationship between serum-bound carbohydrate levels and lymphocyte reactivity. The best correlation was with sialic acid; however, the correlation was not significant. Analysis of the data suggested that study of larger numbers would be required to demonstrate the relationship at a significant level, because of the variability of the lymphocyte reactivity assay. Among the glycoproteins, the alpha-2-HS-glycoprotein correlated significantly (inversely) with lymphocyte reactivity ($p < .01$).

Significance to Cancer Research and the Program of the Institute:

In this study, each of the protein-bound carbohydrates was shown to be related to tumor burden in patients with solid malignancies and was shown to

be a better correlate of tumor burden than CEA. Glycoproteins having a high content of sialic acid showed similar correlations. These observations, combined with the inverse correlations with lymphocyte reactivity in vitro indicate that further studies of larger groups of solid malignancies of well defined histologic types (e.g., squamous carcinomas, adenocarcinomas, melanomas, and sarcomas) are warranted because of this preliminary data indicative of correlation with tumor burden. Demonstration of a correlation in individual patients studied sequentially may allow the earlier detection of residual disease and thus the early institution of adjuvant therapy. Conversely, in patients receiving adjuvant therapy on a long-term basis, these assays may assist in determining the effect on subclinical tumor of the adjuvant.

Proposed Course:

Unfortunately, the contractual arrangements with the Armed Forces Radiobiology Research Institute for analysis of the protein-bound carbohydrates and glycoproteins was limited to 79 specimens. The data indicate that additional specimens will be required to define the relationship between these moieties and tumor burden. At the present time the contract is terminated. Should the contract be reinstituted, additional studies as outlined above will be undertaken to determine the adjunctive value of those markers showing a high correlation with tumor burden and immune reactivity (sialic acid, as alpha-1 acid glycoprotein and alpha-2-HS-glycoprotein). The primary goal of the studies would be to determine their value in monitoring the host-tumor relationship in patients receiving adjuvant chemotherapy and immunotherapy after definitive local treatment of solid malignancies.

Publications:

Silverman, N.A., Potvin, C., Alexander, J.C.,Jr., and Chretien, P.B.: In vitro lymphocyte reactivity and T-cell levels in chronic cigarette smokers. Clin. Exp. Immunol. 22:285-292, 1975.

In Press:

Silverman, N.A., Alexander, J.C.,Jr., and Chretien, P.B.: CEA levels in head and neck cancer. Cancer.

Alexander, J.C.,Jr., Silverman, N.A., and Chretien, P.B.: Effect of age and cigarette smoking on carcinoembryonic antigen levels. JAMA.

Smith, H.G., Horowitz, N., Silverman, N.A., Henson, D.E., and Chretien, P.B.: Humoral immunity to herpes simplex viral-induced antigens in smokers. Cancer.

Smith, H.G., Henson, D.E., and Chretien, P.B.: Viral-specific humoral immunity to herpes simplex-induced antigens in patients with squamous carcinomas of the head and neck. Amer. J. Surg.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CA 03811-02 SURG

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Immunotherapy of Animal and Human Sarcomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATOR AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG NCI
OTHER:	G. A. Parker	Clinical Associate	SURG NCI
	S. Schwarz	Bio. Lab. Tech.	SURG NCI
	H. Anding	Bio. Lab. Tech.	SURG NCI
	H. Wexler	Biologist	SURG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	1.5	3.0

SUMMARY OF WORK (200 words or less - underline keywords)

Concurrent studies on the tumor-host immune interactions in animals and humans with sarcomas have been undertaken. The immune response to murine sarcomas has been studied and at least two antigens have been identified on murine sarcoma cells. One of these antigens is a tumor-specific antigen and the other is a cross reactive antigen present also on murine fetal tissue. Similar studies in the human have identified an antigen on tissue cultured normal as well as sarcoma tissue that is reactive with antibodies present in the serum of sarcoma patients. Studies on the serologic dissection of human tumor antigens is in progress. Adoptive immunotherapies are being evaluated.

PHS-6340
(12-75)

Project Description:

Part I. Antigens on Murine Sarcomas

Part II. In Vitro Studies of the Immune Response to Tumors in Patients with Soft Tissue and Bony Sarcomas

Objectives:

To study the humoral and cellular responses of the host to growing sarcomas. To study and characterize tumor-associated antigens on murine sarcomas. To develop methods of immune manipulation to affect the growth of sarcomas in animal and human hosts.

Methods Employed:

Amputation-challenge experiments have been performed to identify a series of immunogenic sarcomas in mice and rats. A variety of serologic assays have been developed to study the antibody response to tumor-associated antigens on these sarcomas. The major humoral assays that have been evaluated are the C1 fixation transfer test, complement mediated cytotoxicity as measured by microcytotoxicity in microtiter plates and Chromium-51 release, and the radioactive isotope hemadsorption assay. Antibodies in tumor-bearing and immunized mice have been studied. Absorption with a variety of autochthonous syngeneic and fetal tissues have been employed to identify the biologic nature of tumor-associated antigens.

Major Findings:

Many immunogenic sarcomas in mice and rats have been identified that do not cross react with each other in vivo in amputation-challenge experiments. Two distinct tumor-associated antigens have been identified on murine sarcomas. One antigen is specific to each individual tumor and does not cross react with other tumors. A second antigen has been identified that is cross reactive among all murine sarcomas thus far studied. This antigen is also expressed on murine fetal tissue during the first and second trimesters of gestation. Antibody reactivity against the shared fetal component can be removed, leaving antibodies specific for each individual sarcoma. It appears that the tumor-specific antigen and not the shared fetal determinant is responsible for in vivo protection in this murine tumor model.

Significance to Cancer Research and the Program of the Institute:

Understanding the biologic and immunologic characteristics of murine sarcomas will provide valuable information for developing rational immune manipulations for control of these tumors. This information will be of value in designing immunotherapies for human sarcomas.

Proposed Course:

This study will be continued during the next year.

Project Description:

Part II. In Vitro Studies of the Immune Response to Tumors in Patients with Soft Tissue and Bony Sarcomas

Objectives:

To study the humoral and cellular responses of the human host to growing sarcomas. To evaluate the effects of therapy on the immune response to these sarcomas. To develop methods of immune manipulation to improve the treatment of patients with soft tissue and bony sarcomas.

Methods Employed:

Tissue culture techniques have been developed to establish in tissue culture tumor and normal skin from patients in the Surgery Branch with soft tissue and bony sarcomas. Immunologic assays have been developed to monitor the immune response of patients to their own tumors. These assays have largely involved measurements of antibody cytotoxicity as measured by microcytotoxicity tests in microtiter plates and Chromium-51 release assays. A radioactive isotope hemadsorption assay has also been evaluated. Adsorption studies have been performed to identify the nature of the antibody response found in tumor-bearing patients to their autochthonous tumors.

Major Findings:

During the past year both tumor and normal skin from 16 patients with osteogenic sarcoma have been established in long-term tissue culture. These cultures have been used to study the immunologic response of these patients to their autochthonous tumors. Antibodies have been identified in tumor-bearing patients that react with their own tumors, as well as their normal skin in culture. Absorption studies have thus far failed to reveal tumor-specific antigen present in osteosarcoma patients. A natural antibody against determinants expressed on both normal and tumor tissue in tissue culture has been identified and appears to be present as a function of the age of the individual.

Serial studies of immune competence have been performed in patients with soft tissue and bony sarcomas and have documented the general immunosuppression resulting from chemotherapy and radiotherapy.

Significance to Cancer Research and the Program of the Institute:

Soft tissue and bony sarcomas in humans have an overall 5-year survival of about 40%. Our studies in the laboratory are aimed at developing immunotherapeutic methods for improving the treatment of patients with these soft tissue and bony sarcomas.

Proposed Course:

This study will be continuing during the next year.

Publications:

Rosenberg, S.A.: Future prospects for immunotherapy. Cancer 36:821-824, 1975.

NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 CM 03812-02 SURG

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (30 character, or less)

Studies with a Chemically-Induced Rat Colon Cancer Model

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. R. Baker	Senior Investigator	SURG	NCI
OTHER:	J. C. Marrone	Microbiologist	SURG	NCI
	E. W. Chu	Head, Cytopathology Section	LP	NCI
	J. Costa	Expert, Viral Oncology & Molecular Pathology	LP	NCI

COOPERATING UNITS (if any)

Laboratory of Pathology, NCI

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

A DMH induced rat colon cancer is being explored as a model of human colorectal cancer. Studies are designed to determine the immunogenicity of the tumor and examine tumor-host manipulations which lead to enhanced resistance to this neoplasm.

PHS-6040

Project Description:

Objectives:

To explore the utility of a chemically-induced colon tumor in a rat system as a model of human colorectal cancer.

Methods Employed:

Two different DMH-induced colon tumors are carried by subcutaneous passage in syngeneic Buffalo strain rats.

These tumors have been established as cell lines in tissue culture and their characteristics assessed by cell karyotyping. These tissue culture tumor cell lines are used as targets in in vitro assays of humorally mediated tumor-specific immunity.

Classical techniques involving tumor amputation and challenge-protection experiments are used to demonstrate in vivo evidence of tumor-specific immunity.

Rats acclimated to extremely low residue diets have been used to study gut transit time with Cr^{51} labelled red blood cells.

Major Findings:

(1) Vivonex (low residue) fed rats have slower gut transit times than chow fed rats.
(2) The kinetics of cell growth for Buffalo DMH 20-30 and Buffalo 46 transplantable colon tumors have been determined. When inoculated intramuscularly, the Buffalo DMH 20-30 tumor metastasizes by both the hematogenous (to lung) and the lymphatic (to retroperitoneal nodes) routes.
(3) Buffalo DMH 20-30 appears to be an immunogenic tumor. Tumor-bearing animals undergoing amputation at appropriate time after tumor inoculation are refractory to subsequent tumor challenge.
(4) Buffalo DMH 20-30 tumor placed in tissue culture gives rise to a cell line which is malignant by karyotype criteria. Marker chromosomes and abnormal modal chromosome number are constant features. These tissue culture cells, however, fail to grow when reinjected into adult or weanling Buffalo rats.

Significance to Cancer Research and the Program of the Institute:

The magnitude of the colorectal cancer problem in man makes this animal model system very attractive as a focus for studying pertinent clinical questions.

Proposed Course:

Several specific studies are planned.

(1) The relationship between tumor induction and gut transit time will be studied in rats acclimated to diets of varying bulk consistency.
(2) The effect of electrofulguration of tumors on immunity will be examined.
(3) Efforts will be made to develop *in vitro* tests of immunity which correlate well with *in vivo* immunity.

Publications:

None

NOTICE OF
INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Study of Neuraminidase as an Immunoadjuvant in the Treatment of Established Mouse Sarcomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. R. Baker	Senior Investigator	SURG NCI
OTHER:	R. J. Spence	Clinical Associate	SURG NCI
	J. C. Marrone	Microbiologist	SURG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Efforts to demonstrate that C3H mice bearing established methylcholanthrene sarcomas could be made to regress their tumors when treated with a vaccine prepared from neuraminidase-mitomycin C treated tumor cells were undertaken. In three separate experiments we have been unable to reproduce this published observation. Other studies designed to examine whether neuraminidase treatment of a cellular immunogen leads to enhanced immunogenicity of the cell are in progress.

Project Description:

Objectives:

The initial objective of this study was to corroborate Simmon's "in vivo" observation that C3H mice bearing established MCA tumors treated with a vaccine made from neuraminidase-mitomycin C treated tumor cells could be made to regress their tumors with resultant prolonged survival. Later, using cells and serum from regressor and progressor mice, studies to sort out the mechanism of this immunotherapeutic manipulation would be undertaken.

Methods Employed:

In vivo studies - C3H mice are subcutaneously inoculated with syngeneic MCA sarcoma. After the primary tumor has reached a certain size, the mice are treated with multiple injections of vaccine at a site distant from the established tumor. The course of the primary tumor is followed by serially measuring its size and following the animals for survival. Appropriate controls are included to permit assessment of the results of immunotherapy.

In vitro studies -- (1) Using Cr^{51} labelled MCA sarcoma tissue culture cells, a humoral microcytotoxicity system has been developed. (2) To assess activity of the neuraminidase preparations, the Warren thiobarbituric acid assay is used to measure the release of sialic acid from suitably treated tumor cells.

Major Findings:

Three separate therapy experiments conducted in a blinded fashion have been performed thus far. Two of the three experiments used live fresh tumor cells to prepare the vaccine, while in the third experiment vaccine was made using the MC-80 tissue culture tumor cells. None of the experiments was able to demonstrate any significant therapeutic efficacy for the neuraminidase-mitomycin C treated tumor cell vaccine.

Two additional experiments representing a fourth and fifth attempt at demonstrating vaccine effectiveness are in progress and still too early to comment on.

MC-43 and MC-80 have been established in vitro as tissue culture cell lines. Both lines produced tumors when reinjected into syngeneic C3H mice. Allogeneic antisera have been raised which are capable of killing these Cr^{51} labelled tissue culture target cells in vitro. Sera from normal C3H mice, MC-80 tumor-bearing C3H mice, as well as sera from C3H mice whose extremity tumors were amputated ten days earlier are being examined for evidence of syngeneic antitumor cytotoxic activity.

In addition to these studies, other experiments are in progress to examine whether neuraminidase modulation of cell surface determinants enhances the immunogenicity of the cell. C3H mice were immunized with neuraminidase treated C57BL6 splenic lymphocytes or heat-inactivated neuraminidase treated

C57BL6 splenic lymphocytes. The kinetics of anti-H2 immunity were studied by measuring in vitro antilymphocyte cytolytic activity of sera from animals inoculated one, two, three, four, or five times with the above cells. The crisscross experiment was also done immunizing C57BL6 mice with neuraminidase or heat-inactivated neuraminidase treated C3H splenic lymphocytes. In both experiments, neuraminidase treated splenic lymphocytes resulted in no higher level of anti-H2 immune reactivity than did immunization with the heat-inactivated neuraminidase treated splenic lymphocytes.

Significance to Cancer Research and the Program of the Institute:

Neuraminidase treatment of tumor cells is being used to prepare antitumor vaccine in at least two human immunotherapy trials. Independent verification of its therapeutic efficacy in the mouse model system, in which it originally was shown to be active, would lend much encouragement to the aggressive pursuit of these studies in the human clinical situation. Further study of the murine immunotherapy model system offers an opportunity to gain critical insights into basic mechanisms which might well prove exploitable in managing the human clinical problem.

Proposed Course:

The in vivo animal immunotherapy experiments will be pursued to the conclusion of the studies in progress. If these experiments, like the first three, are negative, the results will be reported and work with neuraminidase tumor vaccine terminated.

Publications:

None

NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 CM 03817-02 SURG

PERIOD COVERED:
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cryopreservation of Lymphocytes and Parathyroid Glands

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED IN THE PROJECT

PI:	H. F. Sears	Senior Investigator	SURG NCI
OTHER:	S. A. Rosenberg	Chief of Surgery, NCI	SURG NCI
	G. S. Leight	Clinical Associate	SURG NCI
	G. A. Parker	Clinical Associate	SURG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

Lymphocytes from both normal volunteer patients and cancer patients are cryopreserved and stored for varying lengths of time. These are recovered and used in assays of cellular immune function and compared to those same assays that were done on the fresh cells. The results using normal volunteer cells have shown an ability of the cryopreserved cells to maintain a function in mitogen stimulation assays and mixed lymphocyte response assays that is similar to the fresh cells. The use of simultaneous cultures has decreased day-to-day variability over that which is seen in individual daily assays. Current methods of cryopreservation may alter the function of cells obtained from cancer patients receiving combined modality therapy. This is being evaluated. Parathyroid tissue has been frozen and stored for 1-9 months and reimplanted into the parathyroidectomized autologous animals. These endocrine tissues have been able to sustain life, normal serum calcium levels, and produce parathormone after reimplantation.

Project Description:

Part I. Cryopreservation of Lymphocytes

Part II. Cryopreservation of Endocrine Tissue

Project Description:

Part I. Cryopreservation of Lymphocytes

Objectives:

We desire to provide investigators with immunocompetent cells that can be stored for prolonged periods and retain all the characteristics of these cells in their initial state. By optimizing cryopreservation techniques, lymphocytes will survive and function after liquid nitrogen storage. Cryopreserved cells obtained from normal volunteers react as expected. A method of storing lymphocytes from cancer patients undergoing combined modality therapy that retain all the characteristics of their fresh state is desired.

Methods Employed:

Human lymphocytes have been separated from normal volunteers' blood, as well as the blood of cancer patients having melanoma, osteogenic and soft tissue sarcomas. These patients are undergoing combined modality therapy as part of protocol studies. The normal volunteers have had their blood harvested multiple times over the course of 1-2 weeks. The lymphocytes are separated and some of these are used in assays immediately as control or fresh cells, the rest are cryopreserved. Some of these are immediately thawed and cultured with the fresh cells in the test assays, while others are stored for varied lengths of time of 1 week to 6 months, thawed and cultured in comparable assays as simultaneous cultures. Dimethyl sulfoxide (DMSO) and human serum were used as cryoprotectants. Reproducible results could not be obtained when serum was eliminated from either the cryopreservative media or the recovery media. Seven and one-half to 10 vols. DMSO were found to be the optimal concentrations in agreement with other published reports, and the arrival of the Planer Biologic Freezer in mid-year greatly facilitated the controls rate freezing which had been accomplished previously by laborious multiple timed changes of liquid nitrogen flow-rate.

The techniques involved in the thawing and recovery of cryopreserved lymphocytes have been found to be critical and the accepted rapid thaw with slow dilution of DMSO concentration has been modified by two short culture periods during the dilution. Electron microscopy of the membrane before, during, and after this period are being evaluated.

Viability and function assays of cells frozen on cancer patients are done as simultaneous assays for the entire longitudinal period covering the spectrum of treatment and response for the individual patient. These results are compared to the assays done on the fresh material as individual cultures, at the time it was obtained.

Major Findings:

Optimized techniques of freezing were found to be similar to the reported experience of others in the field with the exception of the added period of culture during the recovery dilution phase. This added step greatly enhanced total yields of viable cells and thereby decreased the possibility of a selective removal of one subpopulation of lymphocytes. Serum is required as a necessary added cryoprotector as it increased yields and decreased variability of recovery in multiple experiments. The normal cells that were frozen and stored were found to react to nonspecific mitogen stimulation (PHA and PWM) as did their unfrozen counterparts. The same was true for the frozen cells used in the 1 and 2 way MLC assays with the exception that the background activity of the unstimulated cells was slightly higher for the frozen cells over this longer culture period. The differences or the variability between the results obtained by the sequentially studied fresh cells were greatly reduced by doing the assay on the whole group together as a simultaneous study. This was true for the mitogen stimulation assays and the mixed culture response assays. During this work it was also demonstrated that both Mitomycin-C treated lymphocytes and irradiated lymphocytes could be cryopreserved and still act as reliable stimulators in these assays.

The preliminary results obtained from the longitudinal study of cancer patients' immune status have revealed that more investigation is necessary before cryopreserved cells can be accepted as the ultimate storage modality. Under some circumstances, frozen cells may be more reactive than their freshly harvested counterparts.

Significance to Cancer Research and the Program of the Institute:

Adequate cryopreservation techniques will provide the investigator with stored material for assays of cellular immunity and tumor immunobiology.

Proposed Course:

Continued investigation of the long-term storage of cryopreserved cells and their ability to function in these assays. Patients undergoing protocol-directed chemoimmunotherapy will be assayed with these frozen stored cells.

Project Description:

Part II. Cryopreservation of Endocrine Tissue

Objectives:

Preservation of human endocrine tissue for study and transplantation as an aid to the therapy of multiple endocrinopathies and other types of tumor surgery is the primary goal of this study.

Methods Employed:

Nineteen mongrel dogs have had their parathyroid glands removed, identified by histologic examination, and cryopreserved and stored for varying lengths of time from 1-9 months. After storage these glands are reimplanted in the autologous host and the last parathyroid gland is removed. After function is demonstrated by rising serum calcium levels, the graft bed is explored, serum parathormone (PTH) levels are determined in the venous effluent blood and compared to the systemic levels of the test animal. The graft beds are resected for histologic examination. The dogs are observed until they become tetanic or die.

The parathyroid gland tissue is preserved by fine 1 mm mincing of the gland and exposure to a 10% DMSO containing culture media with 20% autologous serum. These small pieces of gland are then frozen at a controlled rate of 1°/minute and stored in the vapour phase of liquid nitrogen. The thawing is accomplished by immersion in a 40°C water bath, removal after the last ice crystal is dissolved and the DMSO is slowly diluted from the media. The minced pieces are then placed in pockets of a previously defined muscle bed developed so that hemorrhage is avoided.

Major Findings:

Of the 19 dogs that have undergone parathyroid removal, 13 are suitable for evaluation. The cryopreserved and transplanted glands in 10 of these maintained normal serum calcium levels. Of these 10 dogs, 7 developed severe hypocalcemia after the removal of the transplanted tissue. This demonstrated that the autotransplanted tissue was the only functioning parathyroid present. Four control dogs underwent total parathyroidectomy without transplantation and all became hypocalcemic. In the successful autografts, viable parathyroid tissue was demonstrated histologically in the graft beds. Additionally, parathormone was detected by radioimmunoassay in the sera of animals with cryopreserved parathyroids.

Significance to Cancer Research and the Program of the Institute:

This study demonstrates that parathyroid cryopreservation and autotransplantation can successfully restore parathyroid function in a large animal model. Clinically this technique will be useful in the management of parathyroid hyperplasia, total parathyroidectomy and re-operation for hyperparathyroidism.

Proposed Course:

Further investigations will be carried out to develop the best techniques for cryopreservation of both lymphoid and endocrine cells to ensure not only viability but function, providing an accurate assessment of the material in its fresh state.

Publications:

None

NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03818-02 SURG

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cellular Immunity in the Tumor-Bearing Host

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Cohen	Senior Investigator	SURG NCI
OTHER:	J. M. Jessup	Clinical Associate	SURG NCI
	R. B. Herberman	Chief, Lab. of Immunodiagnosis	LID NCI
	J. L. Weese	Clinical Associate	LID NCI

COOPERATING UNITS (if any)

Laboratory of Immunodiagnosis, NCI

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	2/3	1/3

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with tumors in a variety of locations were evaluated for lymphocyte competence. The response of melanoma patients receiving intralesional treatment with BCG or DNCB was measured serially. Patients with head and neck tumors or breast cancers showed decreased PHA responses compared to normals or melanoma patients, and intralesional treatments did not reverse immune depression in patients with multiple cutaneous melanoma recurrences.

Project Description:

Objectives:

The purpose of the current studies was to assess lymphocyte reactivity in cancer patients and in normals, and to then study immune parameters in patients receiving intralesional BCG or DNCB, as described in a previous section on intralesional injection of recurrent cutaneous melanoma.

Methods Employed:

PHA lymphocyte stimulation was performed by plating 200,000 Ficoll-Hypaque separated lymphocytes into 1 µg of PHA and incubating 48 hours. Each well was then pulsed with 2 microcuries of tritiated thymidine. Twenty-four hours later the plates were harvested with a Multiple Automated Sample Harvester and the radioactive cells collected onto glass fiber discs which were then counted in a Packard liquid scintillation counter. Each sample was tested in quadruplicate. PPD stimulation was performed in a similar fashion, using 0.1 cc of intermediate strength PPD in each well instead of PHA.

Direct leukocyte migration inhibition (LMI) assays were performed to investigate whether cell-mediated immune reactions could be detected in response to tumor-associated human melanoma antigens. The antigens used to inhibit migration of leukocytes from capillary tubes were 3M KCl-soluble extracts of different fresh melanomas, with breast and lung cancer extracts as controls. The percentages of circulating lymphocytes which form rosettes with sheep erythrocytes in a 29°C assay were also determined. In addition, patients were tested serially for their response to skin tests with PPD, SKSD, and mumps antigens.

Major Findings:

PHA stimulation index was higher in normals and melanoma patients (52-53) than in patients with cancer of the breast or head and neck (36-39). In patients with recurrent melanoma receiving intralesional injections with BCG or DNCB, the PHA stimulation indices were the same when all samples were considered in two groups, or when individual patients were considered. The same was true of LMI values. PHA values and T rosette levels were decreased in patients receiving intralesional therapy, compared to normals, and limited studies suggest to date that intralesional treatment does not reverse the immune depression of these patients.

Significance to Cancer Research and the Program of the Institute:

Hopefully in vitro immune assays can be developed that will accurately parallel or predict responsiveness to immune therapy. By applying these assays serially in patients receiving potentially immune potentiating treatment, we hope to be able to evaluate the assays and the immune therapy simultaneously.

Proposed Course:

We plan to work toward the goal of continuing to prove or disprove the utility of PHA, LMI and T cell rosette reactivity and skin testing, and to utilize these assays as the patient's immune reactivity is being altered.

Publications:

None

PROJECT NUMBER (DO NOT USE THIS SPACE)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 03821-02 SURG

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (40 characters or less)

Interactions Between Tumor Immunity and Cellular Immunity to Dinitrochlorobenzene

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. M. Jessup	Clinical Associate	SURG NCI
OTHER:	M. H. Cohen	Senior Investigator	SURG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

SUMMARY OF WORK (200 words or less - underline keywords)

Establishment of delayed hypersensitivity to DNCB results in enhanced growth of subsequent tumor. Preliminary results with nude mice and a non-immunogenic congener of DNCB suggest that T cells mediate this enhancement. When tumor inoculation precedes DNCB sensitization, mice are anergic to DNCB challenge 10 but not 7 days after sensitization. We also found that spleen cells of anergic tumorous mice contained DNCB reactive lymphocytes when tested by transfer to normal mice. Tumor-free DNCB sensitized mice did not develop such cells until much later.

Project Description:

Objectives:

To investigate the effect that a cell-mediated immune response to a chemically-defined hapten such as dinitrochlorobenzene (DNCB) may have upon growth of an experimental tumor and what effect that tumor growth may have upon cell-mediated immunity to DNCB. We will use this data in an attempt to develop methods for analyzing the effect that a patient's immune response may have upon his tumor's growth and the possible modulating effect that cell-mediated immune responses to other antigens may have upon a patient's tumor immunity.

Methods Employed:

A model of delayed hypersensitivity reaction was established in BALB/cAnN mice: 2.0 or 0.2 mg DNCB in 0.10 cc of dimethylsulfoxide (DMSO) was injected intradermally or applied topically to mice. These mice along with untreated littermates, and littermates treated with 0.10 cc DMSO, were injected 10 days later subcutaneously in a hind footpad with 0.05 mg DNCB dissolved in 0.05 cc DMSO. Footpads were then measured 24 to 48 hours later and the results expressed as a percent footpad swelling which was calculated by subtracting the size of the uninjected footpads from that of the injected footpad and dividing the difference by the noninjected footpad.

Tumors employed are transplantable tumors which are carried in ascitic form in BALB/cAnN mice. Meth A is a 3-methylcholanthrene induced fibrosarcoma and Ro→1 is a radiation induced lymphoma donated by Dr. T. Aoki of the Virology and Immunology Branch, NCI. In experiments designed to test the effect of tumors upon DNCB delayed hypersensitivity, washed ascites cells were injected subcutaneously in the flank and at various intervals thereafter animals were injected in the opposite flank with either DNCB or DMSO and then tested 10 days later with footpad challenge as described above. In experiments designed to test the effect of hypersensitivity to DNCB upon tumor growth, mice were pretreated with DNCB, 2 mg subcutaneously in one flank and then challenged 10 days later with an injection of Gloria or Meth A subcutaneously in the opposite flank.

Major Findings:

Mice sensitized with 2.0 or 0.2 mg of DNCB had significantly greater footpad swelling than did mice pretreated with DMSO or left untreated but footpad challenged. This footpad swelling was shown histologically to be composed of a predominantly mononuclear infiltrate, was passively transferrable with immune spleen cells but not with immune serum, and was inhibitable by 2.5 mg of cortisone acetate intraperitoneally given simultaneously as the challenge sensitizing injection. In experiments in animals bearing tumors, it was found that animals bearing $1x10^6$ Meth A cells subcutaneously for 10 days or more had suppression of DNCB sensitivity 10 but not 7 days after DNCB sensitization. However, the amount of swelling in such tumor-bearing animals was the same as the amount of swelling seen in normal mice who are unsensitized

to DNCB, indicating that the inflammatory response is intact. Similar results were obtained with RLo→1 lymphoma. Attempts to elucidate the mechanism of depressed delayed hypersensitivity to DNCB have so far been equivocal. Passive transfer of serum from animals bearing tumor for more than 24 days to normal mice yields consistent suppression of de novo DNCB delayed hypersensitivity. Transfer of spleen cells from mice bearing tumors for 16-25 days to normal mice also seems to inhibit DNCB delayed hypersensitivity.

Finally, we have shown that DNCB sensitized tumor-bearing mice have spleen cells capable of transferring DNCB sensitivity 10 days after DNCB sensitization. Tumor-free DNCB sensitized mice don't do so until 21 days after sensitization.

Significance to Cancer Research and the Program of the Institute:

It is hoped that these studies will provide insight into the mechanism by which tumor depresses cell-mediated immunity and, therefore, give insight into potential therapeutic approaches related to the immune system of cancer patients.

Proposed Course:

We propose to investigate the mechanism *in vivo* and *in vitro* by which tumors inhibit DNCB delayed hypersensitivity. Similarly, we will try to determine if it is the immune response per se which causes tumor enhancement in the DNCB pretreatment model and to see if a suppression of *in vitro* responsivity in PHA and MLC would predict the appearance of enhanced tumor growth.

Publications:

In Press:

Jessup, J.M., Felix, E.L., and Cohen, M.H.: Murine dinitrochlorobenzene (DNCB) reactivity: Depression by melanoma, lymphoma and tumor-associated serum factors. Proc. Amer. Assoc. Cancer Res., 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 03822-02 SURG

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Effect of Retinoid Compounds on Murine Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. H. Cohen	Senior Investigator	SURG NCI
OTHER:	B. C. Loyd	Microbiologist	SURG NCI
	E. L. Felix	Clinical Associate	SURG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2/3	1/3	1/3

SUMMARY OF WORK (200 words or less - underline keywords)

Vitamin A inhibits the growth and development of S-91 melanoma in BALB/c mice. The required dose for this effect is 3500 u/day for 5 days. Recently we observed that the Vitamin A treatment can be given after tumor inoculation. This is the first demonstration of the effectiveness of this compound in preventing growth of transplanted tumor cells. Congeners of Vitamin A are also effective, and possibly have an improved toxic/therapeutic ratio.

Project Description:

Objectives:

Immune adjuvants such as BCG and C. parvum have been used prophylactically to inhibit the development of tumors in multiple animal models. Other known adjuvants such as Vitamin A might, therefore, be capable of acting as inhibitors of tumor growth and development alone or in combination with other agents.

Methods Employed:

Syngeneic mice were pretreated with either oral or intraperitoneal Vitamin A for 5 days. They were then challenged with S-91 melanoma. Tumor incidence and growth rates were measured. Studies to determine the exact dosage of Vitamin A required to inhibit tumor growth were carried out by varying the number of days of treatment or the number of units per treatment. To determine the mechanism of action of Vitamin A, experiments were carried out in which the mice were immunosuppressed using steroids and anti-lymphocyte serum. Experiments are now being conducted in systems that are totally histocompatible, and experiments on using Vitamin A after rather than before tumor inoculation have been performed. Less toxic congeners of Vitamin A are being tested, such as retinoic acid. Experiments are being conducted in cooperation with the cancer chemotherapy screening service to test the efficacy of Vitamin A against leukemia L-1210, B-16 melanoma and syngeneic sarcomas.

Major Findings:

Mice pretreated with oral Vitamin A, approximately 3500 units per day, had a significantly lower incidence of tumor development when compared to controls which received normal drinking water ad lib. Mice pretreated with intraperitoneal Vitamin A, 5000 units for 5 days, also had a significantly lower tumor incidence than did the intraperitoneal saline controls. Dose studies showed that at least 15,000 units of Vitamin A pretreatment was required. When animals were pretreated with steroids, there seemed to be no effect on the Vitamin A protection of the mice. At high tumor doses anti-lymphocyte serum abrogated the Vitamin A protection. At low tumor doses the anti-lymphocyte serum significantly increased tumor incidence over the controls and this enhancement of tumor growth was inhibited by Vitamin A. These experiments show opposite effects of Vitamin A and ALS and are consistent with a possible interrelationship between Vitamin A and the immune system. Experiments in a histocompatible system indicate a weak but beneficial effect of Vitamin A on S-91 melanoma. Also when the compound is given after tumor inoculation the same effect is obtained as when given before tumor inoculation in two experiments. The fact that retinoic acid can cause tumor resistance in this system is encouraging because the agent may be less toxic than Vitamin A; however, toxic/therapeutic ratios must be determined.

Significance to Cancer Research and the Program of the Institute:

It is possible that Vitamin A or its analogues will have a similar inhibitory action on some human neoplasms, so that it may be used as an adjuvant to existing treatment modalities.

Proposed Course:

We plan to study congeners of Vitamin A that may be less toxic than the parent compound, and study more syngeneic systems, to have more relevant study models.

Publications:

Felix, E.L., Loyd, B., and Cohen, M.H.: Inhibition of the growth and development of a transplantable murine melanoma by Vitamin A. Science 189:886-888, 1975.

NOTICE OF
INTRAMURAL RESEARCH PROJECT

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Studies of Genitourinary Tissue Transformed by Oncogenic Viruses and by Chemical Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ASSOCIATES
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Javadpour	Senior Investigator	SURG NCI
OTHER:	None		

COOPERATING UNITS (If any)

None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

ABSTRACT (30 words or less - underline keywords)

In vivo and in vitro immunologic cross-reactivity between SV40 virus transformed prostatic tissue and fetal antigens of a syngeneic strain of hamsters (SLH) suggests a similarity between fetal antigens and tumor-associated antigen of prostatic tissue transformed by a DNA oncogenic virus. Attempts were made to isolate and characterize these oncofetal antigens. An immunogenic murine bladder tumor has also been developed to study the various techniques for immunotherapy of bladder cancer. The consistency of tumor induction, its natural history, and its immunogenicity both in vivo and in vitro render this murine bladder tumor a suitable model for immunologic studies. We are attempting to isolate the tumor-associated antigen of this chemically-induced murine bladder tumor.

Project Description:

Part I. Cross-Reactivity Between Fetal Antigens and Virally Transformed Prostatic Tissue

Part II. Immunologic Features of Carcinogen-Induced Cancer of the Urinary Bladder: In Vivo and In Vitro Studies

Project Description:

Part I. Cross-Reactivity Between Fetal Antigens and Virally Transformed Prostatic Tissue

Objectives:

The relationship between embryonic development and neoplasms has been a central focus of cancer research. Certain fetal antigens, absent from normal adult tissue, have been detected in high concentrations in colonic, hepatic, bronchogenic, and testicular cancers. The purpose of this study is to investigate the relationship of fetal antigens to virally-induced prostatic cancer in hamsters.

Methods Employed:

We have studied the cross-reactivity between prostatic tissue transformed by a DNA oncogenic virus and fetal antigens in hamsters. A syngeneic strain of hamsters (SLH) was utilized. Prostatic tissue obtained from SLH hamsters was grown in tissue culture medium and infected with SV40 virus. The ability of immunization with fetal antigen to protect the hamsters against challenge with transformed prostatic tissue was tested. *In vitro* studies were also designed to determine the cytotoxic activity of the fetal-immune sera against cultured tumor cells.

Major Findings:

Hamsters immunized with fetal antigens demonstrated significant resistance to tumor growth. Complement-dependent humoral cytotoxicity of the target cells (SV40 transformed prostatic cells) in microtest plates was used to demonstrate the presence of significant antibody titer in fetal-immunized hamsters. When the immune sera were absorbed with SV40 transformed prostatic tissue, cytotoxicity indices were markedly lower than with the unabsorbed immune sera. The transformed prostatic tissue was found to have an elevated level of tartrate-inhibited acid phosphatase, suggestive of an epithelial origin of the transformed tissue. These experiments demonstrate *in vivo* and *in vitro* cross-reactivity between fetal antigens and tumor associated antigens of transformed prostatic tissue. This study suggests a similarity between fetal antigens and antigens of a specific organ tissue (prostate) transformed by a DNA oncogenic virus.

Significance to Cancer Research and the Program of the Institute:

The demonstration of immunologic similarity between fetal antigens and tumor antigens may have important implication for the design of a rational approach to the immunologic diagnosis and immunotherapy of cancer.

Proposed Course:

We have repeated these experiments in the LVG/LAK strain of hamster, 1516 strain hamster, and SLH hamsters. The three strains of hamsters have revealed essentially the same findings. Further studies will be directed toward an attempt to isolate and characterize the oncofetal antigens in these three strains of hamsters.

Project Description:

Part II. Immunologic Features of Carcinogen-Induced Cancer of the Urinary Bladder: In Vivo and In Vitro Studies

Objectives:

There is substantial evidence in humans for both cellular and humoral immune responses to cancer of the urinary bladder. Experimentally, bladder cancer can consistently be induced in rats, mice, and dogs by N-4-(5-nitro-2-furyl)-thiazolyl formamide (FANFT). Such tumors have a histologic appearance and natural history similar to bladder cancer in humans. The objective of this investigation is to study certain in vivo and in vitro immunologic features of FANFT-induced bladder cancer in a syngeneic mouse system as a possible model for the immunotherapy of human bladder cancer.

Methods Employed:

The tumor utilized in this study was originally induced in the bladder of C3H mice fed a diet of standard laboratory chow containing 0.1% FANFT by weight in our laboratory and designated as MBT-2 tumor. The threshold for tumor growth and the in vivo immunogenicity of the tumor were determined by conventional laboratory procedures. In vitro tumor immunogenicity was detected by a complement-dependent microcytotoxicity assay. Absorption studies were carried out against individual or pooled sera from the amputated immune animals. These immune sera were absorbed against homogenates of C3H/Hen skin fibroblasts or MBT-2 tumor cells prepared from tissue culture lines. The absorbed sera were utilized in cytotoxicity studies and compared with unabsorbed immune and with normal sera. In addition, cross-reactivity of the MBT-2 tumor with fetal antigens was assessed in vivo by challenging mice immunized with irradiated cells from 12 day old fetal mice or with normal adult liver and spleen cells. The challenge injection concisted of 10^4 tumor cells given subcutaneously 14 days after immunization.

Major Findings:

Bladder tumors were induced in over 80% of C3H mice over an 8-10 month period of FANFT administration. An injection of 10^4 viable tumor cells reliably produced a palpable nodule in 80% of syngeneic recipients. Immunization by amputation of the primary tumor consistently protected the animals from a challenge dose of 10^5 tumor cells. Sera from amputated immune animals was cytotoxic against cultured tumor cells compared to normal C3H sera. This cytotoxic activity could readily be absorbed by tumor cells but not by cultured normal C3H fibroblasts. Unlike other experimental tumors we have studied, immunization of C3H mice with fetal cells did not protect against tumor challenge, nor did the sera from fetal-immune animals demonstrate significant cytotoxicity against the cultured tumor cells.

Significance to Cancer Research and the Program of the Institute:

The consistency of tumor induction, its natural history, and its immunogenicity both *in vivo* and *in vitro* render this murine bladder tumor a suitable model for immunologic studies.

Proposed Course:

This immunogenic murine bladder tumor is being utilized to investigate techniques for the immunotherapy of bladder cancer. Additional studies will include attempts to isolate the tumor-associated transplantation antigen and to purify it by affinity chromatography.

Publications:

Javadpour, N., Hyatt, C., and Scardino, P.T.: Fetal antigens' cross-reactivity with transformed prostatic tissue. Surg. Forum 25:98-99, 1974.

In Press:

Javadpour, N., and Hyatt, C.: Common antigens found on fetal cells and viral transformed adult prostatic tissue. Cancer Res.

NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 CM 06650-01 SURG

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (40 characters or less)

Evaluation of Urinary Reflux Using Renal Autotransplantation and a Pyelocystostomy in Dogs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Javadpour	Senior Investigator	SURG NCI
OTHER:	D. N. Danforth	Clinical Associate	SURG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

SUMMARY OF WORK (200 words or less - underline keywords)

Urinary reflux is a common finding in patients with ileal conduits necessitated by radical pelvic surgery, and knowledge of the degree to which this reflux is capable of inducing renal damage would be important in evaluating the postoerative and long-term problems experienced by these patients. To study this problem, a canine model has been developed in which the kidney is autotransplanted to the iliac fossa and urinary drainage accomplished through a pyelocystostomy, allowing free reflux of urine into the renal pelvis under bladder pressures. Renal glomerular and tubular function is then evaluated periodically, by means of inulin clearances and tubular transport of PAH respectively, and the acute and chronic effects of urinary reflux determined. In this manner, a better understanding of the various drainage procedures available following radical cancer operations might be acquired.

Project Description:

Objectives:

To assess the degree to which urinary reflux is capable of inducing renal injury; to evaluate the pyelocystostomy as a possible drainage procedure in human renal transplants in which more conventional drainage procedures (ureteroureterostomy, ureteropyelostomy) are not possible.

Methods Employed:

Kidneys in dogs are autotransplanted to the iliac fossa using standard vascular techniques, and drainage of urine is accomplished by either leaving the ureter in situ (controls) or by constructing a pyelocystostomy. Glomerular function is evaluated at periodic intervals with creatinine and inulin clearances, and tubular function by means of transport maximum (T_m) for PAH. At the conclusion of the experiment the transplanted kidneys will be examined histologically.

Major Findings:

Studies are currently preliminary; however, there does not appear to be any increase in parenchymal damage in those kidneys drained through a pyelocystostomy as determined by the above clearance techniques. This would suggest that pyelocystostomy is a suitable form of drainage for human renal transplants.

Significance to Cancer Research and the Program of the Institute:

Urinary reflux is a common finding in patients with ileal conduits necessitated by radical pelvic surgery. Knowledge of the degree to which reflux is capable of inducing renal damage would be important in evaluating the postoperative and long-term problems experienced by patients with ileal conduits. The pyelocystostomy is potentially an important form of drainage in those patients with ureteral fibrosis from irradiation, or with ureteral tumors, in whom preservation of the kidney is feasible but no conventional anastomosis for urinary drainage is possible.

Proposed Course:

The animals will be evaluated by the above clearance and tubular transport techniques at monthly intervals following transplantation to evaluate the immediate and long-term changes accompanying the pyelocystostomy. Application to human transplantation can then be estimated.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06651-01 SURG

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies of Transplantation Tolerance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. S. Leight	Clinical Associate	SURG NCI
OTHER:	D. H. Sachs	Head, Transpl. Biol. Section	I NCI
	G. M. Williams	Senior Surgeon	SURG NCI
	S. A. Rosenberg	Chief of Surgery, NCI	SURG NCI

COOPERATING UNITS (if any)

Transplantation Biology Section, Immunology Branch, NCI

LAB/BRANCH
Surgery Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

While many advances in transplantation biology have been made possible by the availability of numerous inbred strains of mice, there remains a need for comparable large animal models for organ transplantation. Over the past three years we have developed three strains of miniature swine, each homozygous for a different allele of the major histocompatibility locus (MHC). To accomplish this in a minimum number of generations, a selective breeding scheme was used. Prior to breeding, the original parents were reciprocally immunized with skin grafts and lymphocyte injections. This produced antisera used in a microcytotoxicity assay with appropriate absorptions to type the offspring of this mating. Selective breeding based on serologic typing of the 62 descendants of this original pairing has led to the development of three separate herds, each homozygous at the MHC. Using hyperimmune sera raised by immunizations between members of these three homozygous strains, 138 different cytotoxicity combinations were tested on lymphocytes from 2nd to 4th generation pigs. No reactions inconsistent with serologic specificities predicted genetically were obtained, confirming the segregation of serologically defined alleles. Mixed lymphocyte cultures likewise confirmed the serologic genotyping: between 68 pairs of animals tested, the mean two-way stimulation index was 1.00±0.07 for serologically identical

Project Description:

Objectives:

To develop several strains of miniature swine, each homozygous for a different set of histocompatibility antigens at the major histocompatibility locus (MHC). After documentation of homozygosity, the animals will be used for in vivo experiments in organ and tissue transplantation, as a source of large numbers of cells from which cell surface antigens can be isolated and characterized chemically, and in studies of tolerance to antigens administered to fetal pigs in vitro.

Methods Employed:

Unlike classical inbreeding schemes which require about 20 generations to produce homozygosity, the approach being used with these miniature swine involves selective breeding on the basis of histocompatibility typing of parents and offspring. Prior to breeding, the original parents were reciprocally immunized with skin grafts and lymphocyte injections. This produced antisera used in a microcytotoxicity assay with appropriate absorptions to type the offspring of this mating. Selective breeding based on serologic typing of the 62 descendants of this original pairing has led to the development of three separate herds, each homozygous at the major histocompatibility locus.

Major Findings:

Using hyperimmune sera raised by immunizations between members of these three homozygous strains, 138 different cytotoxicity combinations were tested on lymphocytes from second to fourth generation pigs. No reactions inconsistent with serologic specificities predicted genetically were obtained, confirming the segregation of serologically defined alleles. Mixed lymphocyte cultures likewise confirmed the serologic genotyping: between 68 pairs of animals tested, the mean two-way stimulation index was 1.00 ± 0.07 for serologically identical animals versus 9.59 ± 2.94 for animals with one or two haplotype differences. Skin graft survivals between serologically identical animals was 11.8 ± 0.88 days versus 7.00 ± 0.36 days for animals with one or two haplotype differences.

Significance to Cancer Research and the Program of the Institute:

One of the major problems in the study of cell surface antigens and organ transplantation in human beings is the lack of control of genetic constitution. The use of inbred mice avoids this problem but because of marked size differences, comparison and application of findings to the human situation is difficult. Additionally, it is extremely difficult to obtain sufficient cells and tissues from rodents to permit quantitative chemical characterization of relevant cell surface antigens. For these reasons it is desirable to have animals of size comparable to human beings whose genetic constitution with respect to histocompatibility could be controlled. Furthermore, studies

of transplantation tolerance in experimental systems will be of value in the development of immunotherapeutic approaches to cancer involving the adoptive transfer of immune sensitized cells.

Proposed Course:

The breeding plan will be continued to produce larger herds in each line and some hybrid lines will be established. Transplantation of organ allografts will be performed in order to characterize the immune response to transplantation and the mechanism of enhancement. Studies of the I_a antigen system and the chemical characterization of histocompatibility antigens will be pursued. The possible use of sensitized pig cells in the therapy of human malignancy will also be explored.

Publications:

None

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR BALTIMORE CANCER RESEARCH CENTER

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

July 1, 1975 - June 30, 1976

The Baltimore Cancer Research Center is well into its second year at the University of Maryland Hospital. The clinical operation with the support services of the Clinical Research Pharmacy Service, Microbiology Section, Infection Research Section, and Cell Component Therapy Unit have provided care for approximately 10,000 out-patient visits as well as approximately 10,000 in-patient days. Renovation plans are being completed so as to provide a new, modern and efficient OPD, Intensive Care Unit and Laminar Air Flow Complex, and appropriate laboratory support services. Until the completion of the adjacent laboratory building, the Laboratory of Pharmacology has continued its activities at the USPHS Hospital. Interaction with University of Maryland has taken place on several fronts and the Baltimore Cancer Research Center sponsored a 3 1/2 day symposium on Advances in Cancer Treatment in conjunction with the UMH Continuing Education Program.

Phase I studies in levamisole were completed with a subsequent Phase II evaluation demonstrating the lack of efficacy in the treatment of bronchogenic carcinoma. New Phase I studies in neocarcinostatan and piperazinedione have been started. Phase II studies of intrathecal thiotepa in meningeal leukemia, dianhydrogalactitol in the treatment of brain tumors, thiotepa, vinblastine, CCNU and piperazinedione in renal cell carcinoma, azotomycin in metastatic sarcoma and adriamycin in mycosis fungoides are underway.

Phase III studies have demonstrated a significant advantage for radiotherapy and chemotherapy in stage III Hodgkin's disease in patients who have associated "E" substage of the lung. In addition, cytoxan, adriamycin and VP16-213 has shown an advantage in the treatment of oat cell carcinoma and a combination of streptozotocin, cyclophosphamide, adriamycin and bleomycin has been shown to be active for advanced refractory Hodgkin's disease. Clinical pharmacology has elucidated a major portion of the human biotransformation pathways of adriamycin and a sensitive radioimmunoassay for the anthracycline drugs has been established. Evaluation of drug-drug interaction of streptozotocin in conjunction with adriamycin has indicated alterations in hepatic metabolism.

Control of infectious disease in the cancer patient has been pursued through the activities of the Section of Microbiology in carrying out a wide variety of surveillance and diagnostic studies and by controlled studies of new antibiotics. The Cell Transfusion Program not only provides routine platelet transfusions and granulocytes to aid in the control of infection,

but carries out research in the collection, preservation and administration of these elements.

The Patient Care Pharmacy continues to supply services including drug and allergy histories, unit dose service and automated monitoring of in-patient and out-patient drug therapy. In addition, data generated by all areas makes use of computer facilities utilizing a wide variety of programs including the specially designed Census program. The PROMIS System of Medical Records is being adapted to the cancer treatment area.

Laboratory investigations with the nitrosoureas have elucidated the hydroxylated part of CCNU, demonstrated the extent of DNA damage to the intracerebral rat glioma by use of alkaline sucrose gradients, investigated the repair of DNA damage and examined cellular respiration as a function of nitrosourea treatment. Studies have also been carried out in the structural activity relationship and human biotransformation of adriamycin and daunorubicin including pharmacodynamic studies and the effect of polarity on membrane transport. The growth and differentiation of normal and malignant human cells as modified by hemaphytogglutinin, and the enzymatic metabolism of the anthracycline glycosides has been demonstrated. The mechanism of human lymphocyte responsiveness to suppressive immunogenic viruses and the ability of complement to mediate cross reactive binding of tumor specific antibody to normal determinants has been shown. Lasar cytometry has allowed rapid evaluaiton of cell changes secondary to immunologic or biochemical manipulation. Subclinical immuno complex disease associated with neoplasm has been shown in patients with acute leukemia and Burkett's lymphoma.

Synthetic polynucleotides have successfully altered the survival of high tumor strain (AKR) mice and studies in the nucleolytic activity in several transplantable tumor systems is being carried out. In addition, specific microbial nucleases are being used to hydrolize nucleic acids in order to develop a sequential analysis of RNA through the identification of specific base linkages. The regulation of nucleic acid synthesis and metabolism is being studied in order to define specific alterations associated with malignancy. Marker studies utilizing the polyamines, alpha feto-protein muramidase, CEA and reverse transcriptase are being evaluated to assist in the staging of disease along with studies of the specific factors involved in the regulations of nucleic acid metabolism.

The Baltimore Cancer Research Center has had another highly productive year of integrated programs of laboratory and clinical research. The association with University of Maryland as well as Johns Hopkins University and other hospitals within the greater Baltimore area has strengthened and collaborative activities at both the basic and applied science level are being undertaken. With the reuniting of the Laboratory of Pharmacology with the clinical area during this forthcoming year, a restabilization of the program will take place. In addition, the clinical service will be renovated and will provide a continuum of care for our patient population. The concept of basic and applied clinical and laboratory research in a relatively self-contained unit with through and through geographic continuity remains unique in the field of cancer research and is one of the major strengths of the BCRC.

SUMMARY REPORT

Clinical Branch, Baltimore Cancer Research Center, DCT, NCI
From July 1, 1975 through June 30, 1976

A number of fruitful projects have been undertaken during this fiscal year and many have produced interesting results already. Phase I study of piperazine dione and of neocarcinostatin has been started. A Phase I study of levamisole was completed which lead to a Phase II study of that drug in bronchogenic carcinoma. The results indicate that levamisole has no beneficial effect on the natural history of bronchogenic carcinoma. A phase II study of intrathecal ThioTepa in meningeal leukemia continues. Early results indicate that this agent is effective against meningeal leukemia. Phase II trials of Azacytidine and VP16-213 in adult acute non-lymphocytic leukemia have been completed with the result that both agents have a certain degree of activity against refractory acute leukemia in adults. A Phase II evaluation of 1-Bamnydrogalactitol in brain tumors is under way. Phase II evaluations of ThioTepa, vinblastine, CCNU, and piperazine dione in renal cell carcinoma continues. Additional Phase II studies include studies of azotomycin in metastatic sarcoma and adriamycin in mycosis fungoides. Phase III studies with daunorubicin and cytosine arabinoside in adults with acute non-lymphocytic leukemia have been very rewarding. Seventy percent of such patients irrespective of age achieve a complete remission with that combination of agents.

Phase III combined modality studies of Hodgkin's disease continue to show significant advantage for radiotherapy-chemotherapy in patients with stage III or with cardiac stages associated with E sub-stage of the lung. A Phase III study of cytoxan, adriamycin and VP16-213 for oat cell carcinoma of the lung has produced some of the best data obtained with chemotherapy or radiotherapy for this disease. Approximately 20% of patients have achieved a complete remission with this regimen and it is clear that complete remission is contributing to substantially improve survival. A Phase III study of streptozotocin, cyclophosphamide, adriamycin and bleomycin for advanced refractory Hodgkin's disease has lead to the conclusion that this combination of agents is highly active in patients who are refractory to, or relapsing from, MOPP therapy.

Epidemiological investigations of patients with leukemia and lymphoma have suggested that such patients are often linked by prior social contact with common, healthy, intermediaries. Further evaluation of these data will be undertaken in the next fiscal year.

A randomized comparison of cephalothin plus carbenicillin, carbenicillin plus gentamicin and cephalothin plus gentamycin for the empiric antibiotic treatment of febrile granulocytopenic cancer patients has identified the combination of carbenicillin and gentamycin as superior from both an efficacious and a toxicologic point of view.

We have discovered that fire proofing material applied to the steel beams in the new hospital building in which the BCRC operates contains aspergillus spores. This has been related to the increased incidence of aspergillus infection immediately after moving into this new facility. Consequently, we have undertaken to decontaminate this fire proofing material and to inform others in similar situations of the potential hazzard such fire proofing material represents for granulocytopenic cancer patients.

We have undertaken a study involving the treatment of patients with diffuse histiocytic lymphoma in a laminar air flow room. This protection allows us to deliver larger than usual doses of cytoxan, adriamycin, vincristine and prednisone to such patients. Early

data indicate that the complete remission rate is high with this approach.

Chemo-immunotherapy trials utilizing MER or levamisole in leukemia, lymphoma, melanoma, and oat cell carcinoma of the lung are in progress and have, to date, been less than rewarding.

Studies with granulocyte transfusions in febrile granulocytopenic cancer patients have indicated that these transfusions can, on occasion, be beneficial and life saving. Studies are underway to develop methods for increasing the yield of granulocytes from normal donors and studies are in progress to reduce the toxicity of granulocyte transfusion to the host. Studies with autologous frozen platelets in acute leukemia patients indicate that these platelets can be life saving to such patients who have become refractory to HLA compatible platelets from other donors.

Biologic markers of malignant disease such as muramidase, alpha feto protein, carcinoembryonic antigen, Reagin isoenzyme, and reverse transcriptase have been studied in some detail and the data correlated with the type and extent of malignant disease.

This massive data has been prepared for analysis and analyzed by pilot computer organized problem oriented medical system. Physicians, nurses, pharmacists and other related health care personnel take part in the in-put and retrieval of this data.

Various new pieces of equipment have been evaluated at the Baltimore Cancer Research Center during this fiscal year including the acoustic microscope, which is an instrument that utilizes microwaves to produce an image of a microscopic particle. Early evaluations indicate that this instrument may be useful for the rapid examination of hematologic material.

Participation in oncology programs at the two medical schools in Baltimore and in other hospitals around the community has provided a source of patient material for our studies and has enabled us to bring to the practicing and academic physician in the community, the impact of the therapeutic advances resulting from the DCT program.

New plans for the next fiscal year include, studies in head and neck cancer and colon cancer.

	NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06027-07 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Microbiological Services for Clinical and Research Studies of Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology	CL, NCI
OTHER:	Marcia R. Moody	Research Microbiologist	CL, NCI
	Arthur S. Levine, M.D.	Chief, Ped. Oncology	COP, DCT, NCI
	Norman R. Friedman	Microbiologist	CL, NCI
	Robert C. Good, Ph.D.	Director, Microbiology	Hazleton Labs.
	Robert W. Chambers	Head, Viral Diagnostic Service	Georgetown Univ.

COOPERATING UNITS (if any)
Pediatric Oncology Branch, COP, DCT, NCI, Bethesda, Md.; Hazleton Laboratories, Vienna, Va.; Viral Diagnostic Service, Georgetown University, Washington, D.C.

LAB/BRANCH
Clinical

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 3/4	1 1/2	1/4

Additional man-years are contracted.

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to supply microbiologic diagnostic services of a high quality for the study of actual and potential infection of cancer patients with viruses, bacteria, plasma and fungi. Further objectives are to study the patients' normal flora, food and environment in an effort to curtail acquisition of potentially pathogenic agents and supply support for evaluation of protective environment and various research investigations. A serum bank provides sera from cancer patients for use by all investigators at the BCRC. A total of 24,340 patient samples were cultured for bacteria and/or mycoplasma and fungi during calendar year 1975. All microorganisms recovered were speciated and otherwise identified by serotyping or bacteriophage typing whenever possible. Emphasis was placed on improvement of the recovery of anaerobes and mycoplasma. Development of resistance to commonly used antibiotics is continuously monitored. Additional studies of antibiotic susceptibilities were done with several new antibiotics and susceptibilities to 6 species of Group D streptococcus were also determined. A total of 186 samples were examined for viral isolation and 314 samples for viral antibody titers. Viral recovery rates ranged from 5 to 10%. Approximately 7% of sera had an 8-fold or greater titer rise to one or more viral antigens.

Project Description: Z01 CM 06027-07 CL

The objective of this project is to supply microbiologic diagnostic services of a high quality for the study of the infections caused by bacteria, viruses, mycoplasma, and fungi in cancer patients. Further objectives are to study the patients' flora on admission to the hospital and acquisition of microorganisms from the hospital environment which are potentially pathogenic. Also, routine surveillance cultures of food and the environment are performed to monitor cleanliness in food preparation and effectiveness of cleaning procedures. This project supplies the microbiological support for evaluation of protective environments and various research investigations.

Cultures for bacteria, mycoplasma and fungi were performed by Hazleton Laboratories under Contract NO1-CM-33705 at a cost of $410,263 for 26,140 man hours of contract personnel. During the calendar year, 24,340 examinations were made for aerobic, anaerobic, and fungal flora. Wherever possible, all microorganisms recovered were speciated utilizing standard procedures as well as newer procedures evaluated and agreed upon by the Project Officer and contracting laboratory. Subspecies were determined for epidemiological purposes by serotyping, bacteriocine typing, and bacteriophage typing. Additional studies of antibiotic susceptibilities of various species of Hemophilus and Group D streptococci were performed and susceptibility tests, both disc and minimum inhibitory concentration determinations, were done with several new antibiotics.

A serum bank, which consists of sera collected on a regular basis from cancer patients at BCRC, provides sera for use by all investigators at the Center. The collection is computerized so that the sera is available by patient, disease, date, etc. This sera is used by the Microbiology Service to determine antibody response to infection.

Diagnostic cultures for viruses, and serologic response to viral infections, were performed by the Viral Diagnostic Laboratory of Georgetown Univ. under Contract NO 1-CM-12133 at a cost of $181,360. There were a total of 186 samples examined for viral isolation with a recovery rate varying from 5 to 10%. In addition, 314 serum samples were examined for antibody titer rises with up to 20 viral antigens.

A review of the viral serologic records of 75 cancer patients revealed that 55 had a 8-fold or greater titer rise against one or more viral antigens. Forty-two such rises occurred in patients with acute non-lymphocytic leukemia, 11 in patients with acute lymphocytic leukemia, and 22 each in patients with lymphoma and Hodgkin's Disease. Among these patients, the most frequent titer rise was to Cytomegalovirus (CMV) (36 instances), followed by Herpes simplex (21) and Varicella zoster (VZ) (12), the Parainfluenza group (11), the Influenza group (8), Respiratory syncytial virus (RSV) (5), and the Adenovirus group (4). A review of clinical manifestations revealed CMV to be the most common virus associated with respiratory infections (40%), Herpes simplex was associated with 20%, RSV with 15%, and the Parainfluenza Group with 15%. The remaining 10% were associated with Influenza virus, Adenovirus and V-Z. Eight of these patients had titer rises associated with death. The majority of skin disorders, as expected, were associated with V-Z and Herpes simplex. Two

febrile patients with erythematous rashes had associated antibody titer rises to CMV. Seven patients had simultaneous titer rises. Two of these showed no correlation with the clinical picture, but the rest had respiratory infections.

A total of 355 strains of Streptococcus faecalis, 61 of S. faecium, 104 of S. bovis, 27 of S. equinus, 30 of S. avium and 13 of S. durans were tested for susceptibility to 10 antibiotics. Significant variations in susceptibility to the various antibiotics occurred among these fecal streptococci. S. faecium was significantly less susceptible to erythromycin, gentamicin and carbenicillin than the other species. Most strains of S. equinus were susceptible to erythromycin but less than half of the S. faecium were susceptible to this antibiotic. Most strains of these streptococci were susceptible to ampicillin, vancomycin, furadantin, and chloromycetin. Many strains were only moderately susceptible to penicillin. Knowledge of the specific species allows more accurate selection of antibiotics for treatment of infection caused by these bacteria.

The contracted facilities continue to provide current microbiologic data for support of patients receiving chemotherapy. It provides the microbiologic basis for a number of protocols (such as the Pharyngitis Protocol No. 7504 and the Herpes zoster protocol No. 7506) and research publications. It has provided the data which demonstrated the presence of pathogenic fungal spores in the insulating material on the overhead beams and the back-up for decontamination procedures (see Project No. 06264). All data are retrievable for investigational purposes (see Project No. 06039).

Publications: None

PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06039-07 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Computerized Microbiology Reporting and Data Retrieval Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology	CL, NCI
OTHER:	John W. Gerster	Computer Specialist	CL, NCI
	William L. Robinson	Computer Specialist	CL, NCI
	Richard K.C. Hsieh	Head, HSR	

COOPERATING UNITS (if any) Viral Diagnostic Service, Georgetown Univ. Washington, D.C.; Health Services Research, U.S. Public Health Service Hospital, Baltimore, Md. Hazleton Labs. Vienna, Va.; Tymshare, Inc., Baltimore, Md.

LAB/BRANCH
Clinical

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2/3	1/2	1/6

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to provide a rapid, accurate means of reporting preliminary and final results of microbiologic diagnostic data generated by a distantly located laboratory facility. The final results are permanently stored and the data are retrievable for use by clinicians and microbiologists to perform prospective and retrospective studies. The computer is utilized to summarize final reports to be placed in the patients' charts. Error detection systems and quality control for the microbiologic data, generated by contracted laboratories is provided by computer programs. The data retrieval programs allow retrieval by various parameters for use in patient care, monitoring of endemic and epidemic infectious agents, and various research support efforts. Immediate review of total cultures of an individual patient or a particular species or organisms can be made. A report of unusual results for review and antibiotic susceptibility summary reports are provided on a regular basis. A new preliminary reporting system is under consideration which should allow for increased efficiency in the entering, updating and finalizing of reports as well as providing an error detection system for preliminary report generation.

PHS-6040
(12-75)

Project Description: Z01 CM 06039-07 CL

The objective of this project is to provide a rapid, accurate means of reporting preliminary and final results of microbiologic diagnostic data generated by distantly located laboratory facilities. Preliminary bacterial and fungal results are sent by Dataphone twice daily to the BCRC. Final results are permanently stored on a magnetic disc. Numerous computer programs allow retrieval of data for use by clinicians and microbiologists to perform various research investigations. The computer programs also include error detection systems for quality control of the data generated by the contracting laboratories. A condensed form of the extensive microbiological data, termed a "Summary Report" is printed monthly by the computer and placed in the patient's chart.

Data containing sample identification; i.e., patient's name and hospital number, source of samples and test requested are entered into data files of a timesharing computer system. The contracting laboratory, when the specimens are to be processed, reviews the information and assigns laboratory numbers. Preliminary results are entered and transmitted to the hospital as they become available. As reports become final, they are batch processed at the computer center and final reports are generated. A new preliminary system is under consideration which would allow preliminary data to be entered temporarily into a timesharing system, updating without retyping all identifying information, error detection and corrections when necessary and preliminary report generation whenever new information is entered. Final report data is permanently stored and used as the base for data retrieval.

The Census Program (Report CM 06220), which records the patients' vital statistics, diagnosis, rooms, bed location and attending physician, is utilized by the Microbiology Reporting System to check for errors and to serve as a base for the epidemiology of various microorganisms.

The data retrieval programs have been used for patient care, for monitoring infectious agents and in the support of various research support efforts. Recent examples are retrievals for Hemophilus spp. and Group D Streptococci with their antibiotic susceptibilities, for all body fluids positive for anaerobic organisms, for all Candida spp. and Torulopsis glabrata, for all filamentous fungi, for all anaerobes, and for pateint summaries by disease category.

Publications: None

PROJECT NUMBER (Do NOT use this space)	NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06219-07 CL

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on Cancer Patient Microbial Flora: Acquisition, Colonization and Infection.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology	CL, NCI
OTHER:	Marcia R. Moody	Research Microbiologist	CL, NCI
	Maureen J. Morris	Laboratory Scientist	CL, NCI
	Robert C. Good, Ph.D.	Director, Microbiology	Hazleton Laboratory
	Philip Hartmen, Ph.D.	Prof. of Biology	Johns Hopkins Univ.

COOPERATING UNITS (if any)

Hazleton Laboratories, Vienna, Va. and the Dept. of Biology, Johns Hopkins University, Baltimore, Maryland .

LAB/BRANCH

Clinical

SECTION

Medicine

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 5/6	1 1/2	1/3

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to study the microbial flora of the cancer patient; i.e., to determine acquisition, colonization and subsequent infection. Routine surveillance cultures of patients determine which organisms are acquired. New and unusual organisms are extensively studied. Proteus spp. and Serratia are subspeciated to specifically identify strains. Acquired organisms more frequently cause infection than the original patient flora. Patients placed on the combined antibiotic therapy protocol are monitored for infecting organisms. A comparison of the susceptibilities of P. aeruginosa to four antibiotics was done due to increased resistance to antibiotics in routine use. The results of this comparison demonstrated differences important for patient therapy. A comparison of positive skin cultures taken from patients in the new BCRC setting with those taken in the previous BCRC setting at the PHS Hospital has led to revised procedures for skin care.

Project Description: Z01 CM 06219 CL

The object of this project is to study the microbial flora of the cancer patient, to determine prevalence, acquisition, optimal antibiotic therapy and investigate unusual organisms. The patient's flora is determined on entrance to the hospital by surveillance cultures, and high risk patients are continuously monitored throughout their hospital stay. Unusual organisms are studied extensively to determine their proper classification and pathogenic potential.

Proteus spp. are subspeciated for surveillance purposes by a modification of the Craddock-Watson test for bacteriocine susceptibility. A new more genetically stable set of test strains have been selected and are being utilized. Freezing of these strains in dimethylsulfoxide allowed greater stabilization of typing properties. Sub-typing of the Proteus strains was achieved by Dienes swarm tests. Most frequently, patients are colonized by a single Proteus strain which persists as an exclusive strain for long periods of time in the intestinal tract.

Serotyping of 133 Serratia spp. from 67 patients revealed that 53 of the patients had more than one serotype. Seven strains failed to serotype. The predominant somatic antigens were 01 and 014, the latter having occurred in 29 patients. 014:H12 was the most frequent; i.e., 39 isolates of Serratia marcescens from 15 patients. The rest of the cultures with 014 somatic antigens had a variety of H antigens. The serotyping method allowed demonstration of a single unusual serotype of Serratia as having caused bacteremia in 3 patients within a 24-hour period, thus incriminating blood products as a possible source.

Patients who are placed on the combined antibiotic therapy protocol (No. 7513) are monitored for infecting organisms and minimum inhibitory concentrations are performed. Also a comparison of the susceptibilities of Pseudomonas aeruginosa to the antibiotics; carbenicillin, ticarcillin, gentamicin, and tobramycin, was made due to the emergence of resistance to antibiotics in routine use. A total of 231 strains of P. aeruginosa from 51 patients were resistant to 50 µg discs of carbenicillin or 10 µg discs of gentamicin were retested with discs containing 100 µg of carbenicillin, 10 µg of gentamicin and tobramycin, and 75 µg of ticarcillin. Minimum inhibitory concentrations (MIC's) were performed concurrently by microtiter utilizing the same inoculum. Based on 4-fold differences in MIC's, 74 strains of P. aeruginosa were more susceptible to ticarcillin than to carbenicillin, 31 of which were susceptible to ticarcillin only. Also, 131 strains were more susceptible to tobramycin than to gentamicin, 51 of which were susceptible to tobramycin only. These results not only demonstrate differences in susceptibility patterns for patient therapy, they also show that conclusions drawn from a single disc susceptibility test for an aminoglycoside or penicillin-like antibiotic cannot be substituted for other antibiotics of similar activity.

A review of the 29,742 cultures taken from cancer patients from June 1, 1974 through September 30, 1975 since the BCRC moved to the University of Maryland Hospital, revealed that there were 2,651 positive skin cultures. A similar review of the 36,778 cultures from patients (June 1, 1968 to May 31, 1974), during the period the BCRC was at the U.S. Public Health Hospital,

revealed 2,337 positive skin cultures, a number significantly lower. Furthermore, the axilla cultures since the move had a greater variety of bacterial species per culture than heretofore. These findings led to revised procedures for skin care.

Publications:

1. Kluge, R.M., Standiford, H.C., Tatem, B., Young, V.M., Schimpff, S.C., Greene, W.H., Calia, F.M., and Hornick, R.B. The Carbenicillin-Gentamicin Combination against Pseudomonas aeruginosa. Ann. Int. Med. 81:584-587, 1974.

2. Schimpff, S.C., Greene, W.H., Young, V.M., Fortner, C.L., Jepsen, L., Cusack, N., Block, J.B., and Wiernik, P.H. Infection Prevention in Acute Nonlymphocytic Leukemia. Ann. Int. Med. 82:351-358, 1975.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 00223-07

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Studies on Pseudomonas aeruginosa in a Cancer Research Center

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATOR AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marcia R. Moody, M.S.	Research Microbiologist	CL, NCI
OTHER:	Viola Mae Young, Ph.D.	Head, Microbiology	CL, NCI
	Maureen J. Morris	Laboratory Scientist	CL, NCI
	Norman R. Friedman	Microbiologist	CL, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 1/6	1	1/6

SUMMARY OF WORK (200 words or less - underline keywords)

This study is designed to investigate the influence of various factors, such as antibiotic resistance, genetic characteristics, host immune response, on the incidence and pathogenicity of Pseudomonas aeruginosa in a cancer patient population. A monovalent lipopolysaccharide fraction of type 2 P. aeruginosa is used as an immunogen in C3H female adult mice. Agglutinin and antigen-binding immunoglobulin levels and the class of Ig in the mouse sera were determined. The importance of these antibodies in the control of P. aeruginosa infection is an ongoing study. The kinetics of the antibody response and its regulation will be performed by the modified Jerne plaque method.

In vitro susceptibilities of Pseudomonas spp. other than P. aeruginosa to certain antibiotics have changed significantly from the antibiograms of these species in an earlier study.

PHS 6040

Pseudomonas aeruginosa has become an important pathogen in cancer patients and prevention of infections by this microorganism is essential. This study encompasses the continuing evaluations of P. aeruginosa and its serotypic incidences in these patients as well as the infectious potential of other Pseudomonas spp. The effects of genetic and environmental factors on the epidemiology of P. aeruginosa and its susceptibility to antibiotics as well as the immune response of the host to this bacterium are under study as a means to provide insight into mechanisms by which P. aeruginosa infections can be eliminated.

The incidences of P. aeruginosa types in this cancer patient population will continue to be evaluated on an ongoing basis. A better coverage of these types has been provided by the purchase of antisera against eight more types.

Studies on the phenotypic and genotypic changes in P. aeruginosa has been instituted to aid in the monitoring of the evolution of antibiotic resistance. Because of the potential impact of infectious resistance on the ecology of P. aeruginosa and other species in the hospital, drug resistance factors and attempts at their transfer from one wild type strain to another are being undertaken.

C_3H adult female mice were used to develop an experimental model to investigate the kinetics of Ab synthesis against P. aeruginosa in the immune host. The immunizing agent used was a P. aeruginosa type 2 lipopolysaccharide fraction from Parke-Davis and Co. Sera from these mice were obtained by retroorbital bleedings at specified times and tested for agglutinins with heat-killed P. aeruginosa of type 2. The antigen in serum dilutions in which no agglutination occurred was washed, resuspended, and retested for agglutination in the presence of anti-mouse total immunoglobin, anti-mouse IgM, IgG, and IgG_2 that has previously been abosrbed with P. aeruginosa.

A direct relationship between immunizing dose and the magnitude of antibody response was apparent in groups of mice that had been immunized with 0.1 µg, 1 µg, 10 µg, and 100 µg of the Fisher type 2 lipopolysaccharide fraction; the maximal antibody response was elicited by 100 µg of the immunogen as only sera from mice immunized with the latter 2 doses had any demonstrable agglutinins. These antibody levels peaked at 2 weeks and declined rapidly to a nondetectable level by the 6th week. Upon the administration of a booster dose of 10 µg at week 12, all groups had maximal detectable agglutinins one week post booster with a gradual decrease to the nondetectable levels by 10 weeks post booster. However, corollary studies demonstrated nonagglutinating immunoglobulins that coated the bacterial cell were present in sera of all mouse groups. By day 10, the levels of these antibodies were at least 4-fold higher than the agglutinin levels. There was a gradual decline in the titer levels but the slope of the decline was not as steep and the antibodies never fell to nondetectable levels. Upon secondary immunization, sera from all groups, except the group primarily immunized with 0.1 µg (peaked 2 weeks post booster) showed the maximal response one week post booster. The antibody

levels in the mice immunized originally with 10 µg and 100 µg did not decline from those levels and were 16-fold and 32-fold respectively higher than the agglutinin levels at 10 weeks post booster. The antibody levels of mice immunized with the lower doses were declining by 3 weeks post booster but at 10 weeks post booster, the levels were still 8-fold higher than the agglutinin levels. The classes of immunoglobulins that were responsible for the nonagglutininating antibodies were showed to be mainly IgM in the 1^{o} immunization until the 5th week at which time IgG was the predominant class present. In 2^{o} immunization, the predominant Ig classes were IgG_1 and IgG_2 for the 100 µg group, IgG_2 for the 10 µg and 0.1 µg groups and IgG for the 1 µg group. The role of these nonagglutinating immunoglobulins in the immune response and the kinetics of antibody synthesis are currently under investigation.

A comparison of the antibiograms of Pseudomonas spp. other than P. aeruginosa that were recovered from cancer patients during an earlier study at the PHS hospital with the antibiograms of the same species from the same patient population at the U. of Md. Hospital 3 or more years later has shown that the in vitro susceptibilities to certain antibiotics had chanted. More strains of P. cepacia were resistant to the polymixins, tetracycline, and streptomycin than in the previous study, while more were susceptible to chloramphenicol, kanamycin, and carbenicillin. There were significant increases in P. maltophilia strains that were resistant to chloramphenicol, colistin, and gentamicin with corresponding increases in strains that were susceptible to tetracycline and carbenicillin. There was little change in the responses of P. putida and P. fluorescens to the antimicrobial agents. In the former species, more strains were resistant to kanamycin, and more were susceptible to tetracycline. Fewer strains of P. fluorescens were resistant to streptomycin and to chloramphenicol. Although tobramycin and ticarcillin were not used in the earlier study, responses of Pseudomonas spp. to these agents parallels the responses to gentamicin and carbenicillin respectively. Epidemiological monitoring of Pseudomonas spp. will continue and additional studies will be instituted when warranted.

Publications:

1. Moody, M.R. and Young, V.M. In Vitro Susceptibility of Pseudomonas cepacia and Pseudomonas maltophilia to Trimethoprim and Trimethoprim-Sulfamethoxazole. Antimicrob. Agents and Chemother. 7:836-839, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06254-07 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Research Activities Directed Toward Improvement of Microbiology Laboratory Techniques

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology	CL, NCI
OTHER:	Marcia R. Moody	Research Microbiologist	CL, NCI
	Maureen J. Morris	Laboratory Scientist	CL, NCI
	Norman Friedman	Microbiologist	CL, NCI
	Michael T. Hargadon	Microbiologist	CL, NCI
	Robert C. Good, Ph.D.	Director, Microbiology	Hazleton Labs.

COOPERATING UNITS (if any)
Hazleton Laboratories, Vienna, Va.; Viral Diagnostic Service, Georgetown University, Washington, D.C. 20007

LAB/BRANCH
Clinical

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5/6	2/3	1/6

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to evaluate and implement new methodology whenever it offers an advantage to recovery of microorganisms from cancer patient samples and to develop and standardize methods for performing accurate susceptibility tests including those involving newly developed antimicrobial agents. Tests enabling the detection of Cryptococcus neoformans antigen in the serum and cerebrospinal fluid of cancer patients is now available on a routine basis. A system has been set up to determine phage-types of S. aureus recovered from patients, personnel and environment. An evaluation of questionable or weakly positive coagulase tests for S. aureus is also being done to confirm recent reports in literature that these questionable reactions should not be considered positive. Due to a low yield of viruses from viral cultures, a new system of direct tissue culture inoculation is now being utilized and is being compared to the previous viral holding broth. A new schema has been devised for the differentiation and identification of the saccharolytic pseudomonads other than P. aeruginosa. We are presently attempting to determine the applicability of its determinations under routine use.

Project Description: Z01 CM 06254-07 CL

The objective of this project is to evaluate and implement new methodology in microbiology that might offer an advantage in recovery and specific identification of microorganisms from samples of cancer patients and to develop and standardize methods for performing accurate susceptibility tests for new antibiotics as they become available.

Materials have been obtained from the Industrial Biological Laboratory and evaluated which enable detection of Cryptococcus neoformans antigen in the serum and cerebrospinal fluid of cancer patients. This test is now available on a routine basis.

Staphylococcus aureus is now the chief cause of infection in cancer patients. Staphylococcal phages have been obtained and a phage-typing system set up. All Staphylococcus aureus strains recovered from patients, personnel, and the environment are phage-typed. A computer program has been written to enable tracing various Staphylococcal phage-types by individual patient and according to room location.

An increasing number of Staphylococcal strains produce questionable or weakly positive coagulase tests (the chief differentiating procedure between S. aureus and S. epidermidis). Recent reports in the literature indicate that these questionable reactions should not be considered positive. To confirm these reports, a study has been set up to determine if plasma from different sources give similar reactions, and to compare these results with DNase production, carbohydrate formulation and phage typability. An analysis of these parameters should eliminate the problem of obtaining definitive results.

A review of results obtained from viral cultures revealed that the yield of positive samples was lower than would have been anticipated. To create optimal conditions for viral growth, samples for viral culture are no longer placed in viral holding broth, but samples are regularly placed directly in 2 tubes each of 4 cell lines; i.e., African green monkey kidney, HEP2, WI38, and HEK cells. A comparison of results obtained is in progress.

The utilization of Czapek agar for the growth of fungi has not proven to enhance detection of Aspergillus spp. but will continue to be utilized because its suppression of contaminating bacteria and non-pathogenic fungi allows greater ease and speed in isolation and identification of these organisms.

Of the species of Pseudomonas which are not P. aeruginosa, the saccharolytic pseudomonads are the most important. This group of organisms presents problems in correct species identification, particularly between P. putida and P. fluorescens. A schema has been devised for the differentiation of these bacteria, which includes the use of minimal media (from the method of Stanier) as well as the usual techniques. This schema is now under evaluation to determine its applicability and the correctness of its determinations under routine use.

Publications: Morris, M.J., Young, V.M. and Moody, M.R. An Evaluation of the API Enteric Strip for the Speciation of Saccharolytic Pseudomonads. J. of Clin. Microbiol. In Press.

INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER
NOTICE OF	Z01 CM 06938-02 CL

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Environmental Studies to Prevent Hospital Acquisition of Potentially Dangerous Microflora

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Viola Mae Young, Ph.D.	Head, Microbiology	CL, NCI
OTHER:	Norman R. Friedman	Microbiologist	CL, NCI
	Maureen J. Morris	Laboratory Scientist	CL, NCI
	Marcia R. Moody	Research Microbiologist	CL, NCI
	Robert C. Good, Ph.D.	Director, Microbiology	Hazleton Labs.

COOPERATING UNITS (if any)

Hazleton Laboratories, Vienna, Va.

LAB/BRANCH

Clinical

SECTION

Medicine

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1 1/6	1	1/6

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to survey the patients' environment and the personnel who work in this environment for potentially pathogenic microorganisms. Means are sought to eliminate such organisms. Food items are continuously being surveyed and those containing contamination are eliminated. Protective environments are continuously being monitored in order to protect patients from acquisition of bacteria from the hospital. The environment of a nearby motel, which houses many of our outpatients, is also under routine surveillance. An extensive study of S. aureus recovery from patients, personnel and environment is underway. A new procedure has been established to determine whether patients who carry S. aureus shed these organisms into the environment. Precautions are taken for those patients who do shed to prevent the spread of their organisms. Due to an increase of Aspergillus infections, an investigation was initiated to determine a possible common source. This source was found to be the heavy contamination by fungi of insulating material on overhead beams within the ceiling structure. Decontamination by spraying with Copper-8-quinolinolate is underway. Environmental cultures have been used throughout t investigation to evaluate the procedures.

PHS-6040

Project Description: Z01 CM 06938-02 CL

The objective of this project is to survey the patients' environment; air, food, water, products used in their care, and the personnel who work with them, for potentially pathogenic microorganisms. Methods for elimination of such organisms are evaluated and instituted when successful. Routine surveillance procedures are utilized for the monitoring.

As Staphylococcus aureus is now the chief cause of infection among patients at the Baltimore Cancer Research Center, considerable effort has been placed on studies of this bacterium. Personnel of BCRC were cultured (nose, hands, and beards or moustaches). Of 127 persons cultured, 24 (18.8%) carried S. aureus (19 nose, 7 hands, 1 beard). On re-culturing the positive carriers, 16 persons were positive (16 nose, 3 hands). These individuals were informed of their carrier status and preventive measures were taken. Three months later, 75 individuals in closest patient contact were surveyed and 17 carried S. aureus (10 nose, 9 hands). On re-culturing the carriers, 7 persons were positive (4 nose, 1 throat, 2 hands). Five months later, nose cultures only were performed on 101 persons. 23 carried S. aureus. Re-culturing revealed only 7 nasal carriers. A new procedure has been established to determine whether patients who carry S. aureus shed these organisms into the environment. Additional precautions are taken for those patients who do shed to prevent spread. Heavy shedders, such as patients with Mycosis fungoides, who become S. aureus carriers are kept under suitable isolation precautions. Monitoring of the environment has revealed that S. aureus phage types not carried by the patients are present in their rooms, on floors, tables, etc. Mop strands have revealed these organisms also. As a result, cleaning procedures are under review and cleaning personnel are being surveyed.

Demonstration of an increase in Aspergillus infections among the patients led to an investigation of possible sources. Extensive culturing of air supply systems did not reveal a common source. However, the presence of numerous fungal spores on the top of ceiling tiles led to the discovery that insulating material on overhead beams was heavily contaminated with fungi. Air ducts in the South clinic area (older section of the hospital with air-conditioning units rather than a central source of filtered air) also contained many pathogenic Aspergillus spores. At the recommendation of a Committee assigned to handle this problem, copper-8-quinolinolate was successfully utilized to destroy fungi and fungal spores in a test area and presently is being used further to similarly decontaminate the whole cancer patient area. Environmental cultures have been used throughout the investigations to delineate the problem and evalute the procedures.

The evaluation of protective environments (Laminar-air-flow rooms, Med-Assist air filtration units and reverse isolation rooms) continues. Over 30 sites in each room is cultured weekly. These cultures serve to monitor proper decontamination procedures and patient shedding of microorganisms as well as to provide evaluation data for the sources of possible organism acquisition by the patients.

A study designed to evaluate possible importance of droplets produced by flushing toilets as a source of patient contamination was undertaken. A toilet

was seeded with 4.3 and 4.8 x 10^7/ml Escherichia coli. The number of these organisms recovered at seat level was 27. Furthermore, a Pseudomonas putida which was susceptible only to Polymixin and Colymycin was recovered from the toilet water. Organisms with similar susceptibilities have been isolated from toilet rims of 7 patient rooms. Several patients have died from organisms, apparently hospital acquired, which have had similar susceptibility patterns. Betadyne is now poured into toilet bowls and sink drains to decontaminate them following each use.

The foods which are seldom, if ever, contaminated have been determined by extensive cultures and are now in routine use. As new or different foods are requested by patients, they are cultured to determine that they are free from bacterial contamination.

Housing for cancer patients receiving chemotherapy who do not require hospitalization is provided in a nearby motel. The environment of the rooms provided for such patients has been extensively cultured and has proven to be free, thus far, from potentially dangerous microflora.

Publications:

1. Aisner, J., Schimpff, S.C., Bennett, J.E., Young, V.M., and Wiernik, P.H. Aspergillus Infections Among Patients with Cancer: An association with fire-proofing materials in a new hospital building. J.A.M.A. 235:411-412, 1976.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM-06217-07 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Prevention of Infections in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATOR AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Senior Investigator	CL NCI
OTHER:	J. Aisner	Senior Investigator	CL NCI
	M. Brouillet	Infection Control Nurse	CL NCI
	D.M. Hahn	Clinical Associate	CL NCI
	P.H. Wiernik	Chief, Section of Medical Oncology	CL NCI

COOPERATING UNITS (if any)

V.M. Young Research Microbiology Section
C.L. Fortner Patient Care Pharmacy

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9	6.5	2.5

SUMMARY OF WORK (200 words or less - underline keywords)

Infection remains as a major cause of both morbidity and mortality for patients with various types of cancers. The risk of infection increases as new techniques are introduced into medical practice and as greater numbers of patients are treated with more intensive cytotoxic and immunosuppressive therapy. It is therefore essential in the modern practice of oncology that definitive approaches be designed and evaluated to reduce the opportunities for infection. At the Baltimore Cancer Research Center an intensive infection surveillance program assesses the ongoing infectious disease status of the patients and the Center as a whole. From this data base have grown appropriate basic infection control policies and specific research approaches including evaluation of laminar air flow rooms, oral nonabsorbable antibiotics and staphylococcal colonization eradication each of which has been scientifically proven to be of value in infection control. The net result has been a very substantial reduction in infections among patients at this institution.

Infection remains as a major cause of both morbidity and mortality for patients with various types of cancers. The risk of infection increases as new techniques are introduced into medical practice and as greater numbers of patients are treated with more intensive cytotoxic and immunosuppressive therapy. It is therefore essential in the modern practice of oncology that definitive approaches be designed and evaluated to reduce the opportunities for infection.

A) Surveillance - The cornerstone of an infection control program is an accurate understanding of the infections presently occurring including the sites and etiologic agents, apparent predisposing factors, routes of acquisition of hospital organisms, and changing patterns of morbidity and mortality. At the Baltimore Cancer Research Center this is accomplished by the daily rounds of an Infection Control Nurse, twice weekly infectious disease rounds, daily evaluation of all infected or potentially infected patients, and an extensive program of both patient and environmental microbial culturing. The data derived is summarized regularly and utilized to adjust, on a continuing basis, the programs in infection control.

B) Basic Infection Control Procedures - During the past year a Policy Manual has been developed with enforcement persued by the Infection Control Nurse. The following procedures are standard: 1) urinary, intravenous and hyperalimentation catheters are avoided except under extenuating circumstances, 2) all intravenous solutions are prepared in laminar flow hoods, all bottles and tubing are replaced daily, and butterfly needles are changed every other day, 3) personnel hygiene is emphasized including handwashing and the use of special germicidal hand lotions, 4) all inpatients are seen regularly by the Infection Control Nurse for discussion of preventive techniques, 5) results of patient and environmental surveillance cultures are utilized to direct specific control techniques. These techniques have been definitely proven by clinical investigation to reduce nosocomial infection.

C) Oral Nonabsorbable Antibiotics - Controlled trials at the BCRC have indicated the definitive usefulness of these drugs to reduce the incidence of severe infection including perianal lesions, colonic lesions, pharyngitis, and pneumonia with consequent reduction in gram-negative bacillary bacteremias and infectious death in the granulocytopenic patient with acute leukemia. In conjunction with other techniques, the incidence of bacteremia in these patients has dropped from 8.9 per 1000 patient days to 1.7/1000 days with the remainder occurring almost exclusively in late stage patients who could no longer tolerate oral therapy.

D) Reverse Isolation - Previous investigations have proven the usefulness of laminar air flow rooms to reduce the incidence of infection in acute leukemia, but it is not established when air filtration per se is of importance nor whether the expensive technology of LAF rooms is essential. At the BCRC, patients with acute nonlymphocytic leukemia are randomly allocated to no reverse isolation, reverse isolation without air filtration, or reverse isolation with filtered air. Thirty-five patients have been randomized to

date with, as yet, no significant difficiencies in severe infections or bacteremias between the 3 groups. The critical factor in infection control appears to be the oral nonabsorbable antibiotics.

E) Aspergillus pneumonia - After BCRC moved to a new hospital location in 1974, an increase in respiratory tract Aspergillus sp infection was documented unrelated to changes in disease therapy or supportive care. An extensive search revealed that the steel-beam fireproofing material was contaminated with various fungal organisms including Aspergillus flavus, fumigatus, and niger. A fungicidal agent, copper 8-quinolinolate, was evaluated by spraying all insulation and false ceiling tiles in a test room: Insulation material and false ceiling cultures revealed fungi before but not after application and air sample colony counts after application were reduced compared to the control period. As a result, all insulation material and involved false ceilings will be treated with this compound in hopes that Aspergillus infection will be reduced substantially.

F) Staphylococcus aureus infection - Infection with S. aureus tends to occur in the patient who becomes colonized in nose, throat or skin and then becomes granulocytopenic. Routine surveillance cultures document colonization and permit attempts to eradicate the organism before infection can occur. A combination of rifampin plus cloxacillin has been under evaluation because of the intracellular activity of rifampin (where many strains of S. aureus are thought to persist). Among the first 15 patients treated with this combination for 5 days, 14 had total eradication of S. aureus from all cultures. The program continues with documentation of fewer S. aureus infections during the past 8 months compared to the previous year before this approach was begun.

Publications:

Schimpff, S.C.: Laminar air flow room reverse isolation and microbial suppression to prevent infection in patients with cancer. Cancer Chemother. Rep. 59: 1055-1060, 1975.

Aisner, J., Schimpff, S.C., Bennett, J.E., Young, V.M., and Wiernik, P.H.: Aspergillus infections in cancer patients. Association with fireproofing materials in a new hospital. JAMA 235: 411-412, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06230-07 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Diagnostic Laparotomy and Splenectomy for Staging Hodgkin's Disease.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI : P.H. Wiernik Head, Clin. Br. CL NCI
J.A. Levi Sr. Clin. Assoc. CL NCI

Other : J. Sutherland
J. A. Singer
M. Cooper
C. Diggs

COOPERATING UNITS (if any)
Pathology, Tumor Surgery, and Radiology and Nuclear Medicine Departments, University of Maryland Hospital, Baltimore, Md.

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of this study have been to determine the value of staging laparotomy on evaluating the accuracy of the extent of abdominal disease at the time of initial evaluation and what influence this has on the initial therapy for patients with early stage Hodgkin's disease. 175 patients with Hodgkin's disease have undergone staging laparotomy and splenectomy and this has revealed that the pre-operative assessment of the extended abdominal involvement was incorrect in approximately 40% of these cases. The prediction of splenic involvement by Hodgkin's disease was incorrect in one-third of the patients and approximately one-third of abnormal lymphangiograms could not be confirmed at laparotomy and a number of cases of involvement of nodes in the porta hepatic region outside the usual radiation therapy ports were discovered. A small number of patients were also found to have documented liver involvement which had not been suspected by pre-operative evaluation. These findings have altered the therapeutic approaches significantly in the number of patients. It has also been determined that patients presenting with supradiaphragmatic Hodgkin's disease have occult disease limited to the spleen, splenic hilum and celiac axis in most cases in which abdominal tumor documented at laparotomy. The results of these studies have been important in the subsequent design of our further therapeutic protocols for the treatment of stages IA to IIIA Hodgkin's disease.

PHS-6040

Project Description: Z01 CM 06230-07 BCRC

Accurate determination of extent of lymphomatous involvement with Hodgkin's disease is important for the individual patient's prognosis and therapy. Valid assessment of the efficacy of different therapeutic modalities used in a population with Hodgkin's disease is also dependent upon a precise localization of disease prior to therapy. The purpose of this study is to determine more accurately the extent of abdominal disease at the time of diagnosis and prior to therapy by performing diagnostic laparotomy.

Analysis of 175 patients with Hodgkin's disease who have undergone staging laparotomy and splenectomy has revealed that pre operative assessment of the extnet of abdominal involvement was incorrect in approximately 40% of the cases. Prediction of splenic involvement by Hodgkin's disease was incorrect in 1/3 of the patients, approximately 1/3 of abnormal lymphangiograms could not be confirmed at laparotomy, and a number of cases of involvement of nodes in the porta hepatis region outside the usual radiation therapy ports were discovered. Occult hepatic involvement was discovered in some cases in whom liver function tests and physical examination were normal, and others suspected of having liver involvement were shown th have disease confined only to lymph nodes. These findings altered therapeutic approaches significantly in many patients.

It was discovered that patients presenting with supradiaphramatic Hodgkin's disease had occult disease limited to the spleen, splenic hilum and celiac axis nodes in most cases in which abdominal tumor was documented at laparotomy. Extranodal involvement was not seen in any of the 85 patients with supradiaphragmatic clinical stage I or II Hodgkin's disease, and most patients would have had all apparent disease adequately treated had extended field radiation therapy been administered without performing staging laparotomy. Therefore, we are currently randomizing patients who present with nodal disease above the diaphragm who do not have the systemic "B" symptoms of Hodgkin's disease to undergo extended field radiation therapy on the basis of their clinical stage without performing laparotomy, or alternatively to undergo surgical staging with modification of therapy based on the documentation of abdominal Hodgkin's disease. Although a number of patients have been entered on this study, it is too early for evaluation at this time.

Patients who present with a suspicion of abdominal involvement undergo laparotomy prior to the initiation of therapy because of the relatively high risk of involvement of nodal sites outside standard radiation therapy ports, or extra nodal involvement, particularly of the liver.

Publications:

1. O'Connell, M., Schimpff, S., Kirschner, R., Abt, A. and Wiernik, P.: Epithelioid granulomas in Hodgkin's disease - A favorable prognostic sign? JAMA 233: 886-889, 1975.

2. Schimpff, S., O'Connell, M., Greene, W. and Wiernik, P.: Infections following splenectomy in ninety-two patients with Hodgkin's disease: A clinical review. Amer. J. Med. 59: 695-701, 1975.

3. Schiffer, C., Levi, J. and Wiernik, P.: The significance of abnormal circulating cells in patients with Hodkin's disease. Brit. J. Haematol. 31: 177-183, 1975.

FOR T... INFORMATI... EXCHANGE ... DEPARTMENT OF ...
PROJECT NUMBER (DO NOT use this space) HEALT... ...
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-CM 06259-04 CT,

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (50 characters or less)
Extension of the Computerized PROMIS System into the Cancer Treatment Area

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATOR AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	:	R. J. Esterhay	Sr. Investigator	CL NCI
Other	:	P. Chang		CL NCI
		A. Hammet	R. N.	CL NCI
		W. R. Grove	Pharmacist	CL NCI
		C. L. Fortner	Pharmacist	CL NCI
		W. L. Robinson	Res. Prog.	CL NCI
		C. A. Rulen	A.R.T.	CL NCI
		V. M. Young		CL NCI
		P. H. Wiernik	Head, Clin. Br.	CL NCI
		M. D. Walker		CL NCI

COOPERATING UNITS (if any)
PROMIS Laboratory, Department of Medicine, University of Vermont, Burlington, Vermont 05401

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	4	0

SUMMARY OF WORK (200 words or less - underline keywords)

The Baltimore Cancer Research Center (BCRC) has developed cancer guidance displays (treatment protocols) on the computerized Problem Oriented Medical Information System (PROMIS) in Burlington, Vermont at the University of Vermont School of Medicine, with the aid of a communication linkage between BCRC and PROMIS. The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the four phases of medical action of the Problem Oriented Medical Record. The problem defining sequence determines the specific plan, and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. The protocol couples the health provider to the protocol specific antineoplastic drug displays which provide feed back loops to the patient's problem list and outstanding orders. It is through the use of these explicit informational and directional displays that the health provider can couple learning with doing while gathering information and treating patients' cancer problems.

PHS-6040
(12-75)

Project Description: Z01 CM 06259-04 CL

To extend the Computerized Problem-Oriented Medical Information System (PROMIS) to the cancer treatment area to allow the development of structured input ("cancer displays") for coupling learning with doing for all personnel who care for individual cancer patients entered on problem specific plans (research protocols).

Methods Employed:

1. The computer terminal being used for this purpose is a touch-sensitive cathode ray tube display device with electrostatic printer similar to those in use in Burlington, Vermont, where the computer is located. BCRC has been responsible for the installation since February, 1972. All programming and operation of the computer system have been done at the University of Vermont.

2. The basic components of the remote terminal at the BCRC are the same as the local terminals in Burlington, Vermont: display screen, display memory, keyboard, Digiscribe, and communication link to the computer in the PROMIS Laboratory, Burlington, Vermont.

3. The BCRC staff has developed anti-neoplastic drug displays which have been integrated with the displays already on file in Burlington, Vermont, as part of the PROMIS system. Currently over 30,000 displays have been developed by the PROMIS group. The drug information sequences have been up-dated and new sequences have been added.

4. Problem-specific display plans for acute non-lymphocytic leukemia and non-Hodgkin's lymphomas have also been developed which includes a series of protocols for treating these specific problems. The protocol displays couple the user to the protocol specific information and drug ordering display sequences. The problem formulation displays for chronic lymphocytic leukemia and Hodgkin's disease have been developed. Displays for problem-oriented reporting of bone marrow findings are being developed. Problem-determined plans for conventional as well as protocol treatment are under development for chronic lymphocytic leukemia and Hodgkin's Disease.

The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the four phases of medical action of the Problem-Oriented Medical Record. The problem defining dequence determines the specific plan, and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. The protocol couples the user to the protocol specific anti-neoplastic drug displays which provide feedback loops to the patient's problem list and outstanding orders. It is through the use of these explicit informational and directive displays that the user can couple learning with doing while gathering information and treating patients' cancer problems.

NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 CM 06263-05 CL

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Protocol for Staging and Treatment of Lymphosarcoma and Reticulum Cell Sarcoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Head, Clinical Branch	CL NCI
Other:	John A. Levi, M.D.	Clinical Associate	CL NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

Medicine

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of this study have been to assist the value of surgical staging in patients with apparently localized non-Hodgkin's lymphoma and whether or not adjuvant chemotherapy will improve the treatment results in patients with localized non-Hodgkin's lymphomas who receive initial irradiation therapy. Patients with clinically localized stage I and II non-Hodgkin's lymphoma are subjected to a staging laparotomy and following this evaluation, the patients are found to have pathologic stages I and II disease are then randomized to receive either extended field irradiation alone or extended field irradiation followed by six courses of chemotherapy utilizing cytoxan, vincristine and prednisone. Twelve patients whose staging laparotomies were entirely negative have been randomized to radiation therapy with or without adjuvant chemotherapy to this time. Relapses have been seen in both study groups. Too few patients have been accrued at this time to determine the value of adjuvant chemotherapy in treating localized malignant lymphomas.

PHS-6040

Project Description: Z01 CM 06263-05-CL

1. To assess the value of surgical staging in patients with apparently localized lymphomas.
2. To determine whether adjuvant chemotherapy will improve treatment results in patients with localized lymphomas treated with radiation therapy.

Patients with malignant lymphomas which are apparently clinically localized (stage I or stage II) undergo staging laparotomy to detect occult foci of intra-abdominal tumor. Patients with negative laparotomy findings (pathological stage I or II) are then randomized to receive either: (a) radiation therapy alone or (b) radiation therapy followed by 6 courses of combination therapy utilizing cytoxan, vincristine, and prednisone. Patients who are found to have at least stage III disease are treated with combination chemotherapy on a different protocol.

Several patients with apparently localized supradiaphragmatic malignant lymphoma have been found to have at laparotomy an occult disease below the diaphragm which would not have been properly treated had local therapy been given on the basis of clinically apparent disease only. Twelve patients whose staging laparotomies were entirely negative were randomized to radiation therapy with or without adjuvant chemotherapy. Relapses have been seen in both study groups. Too few patients have been accrued at this time to determine the value of adjuvant chemotherapy in treating localized malignant lymphomas.

There is evidence that truly localized malignant lymphomas are radiocurable if high dose radiation is given to all sites of involvement. On the other hand, such approaches would not be expected to be effective in patients with disseminated disease. Employing staging laparotomy as an additional diagnostic test to determine the extent of disease, therefore, may allow better patient selection for attempts at curative radiation therapy. In addition, it should be determined whether the addition of adjuvant chemotherapy will significantly prolong disease free and overall survival.

Publications:

1. Levi, J., O'Connell, M., Murphy, W., Sutherland, J. and Wiernik, P.: Role of ^{67} Gallium Citrate Scanning in the Management of Non-Hodgkin's Lymphoma. Cancer 36: 1690-1697, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06264-07 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Evaluation of the Types and Causes of Infectious Diseases in Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Senior Investigator	CL NCI
OTHER:	J. Aisner	Senior Investigator	CL NCI
	M. Brouillet	Infection Control Nurse	CL NCI
	D.M. Hahn	Clinical Associate	CL NCI
	P.H. Wiernik	Chief, Section of Medical Oncology	CL NCI

COOPERATING UNITS (if any)

V.M. Young Research Microbiology Section

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	.50

SUMMARY OF WORK (200 words or less - underline keywords)

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aims of this project are to catalogue and identify the infections, identify the predisposing factors, and evaluate new techniques for more rapid and/or accurate diagnosis of infection. This program has been in progress for over 5 years and has allowed meaningful evaluation of the types of infection occurring in different types of tumor patients. Further, specific problems have been identified such that appropriate intensive preventive measures could be devised and enforced with consequent marked reduction in infection morbidity.

PHS-6040

Project Description:

Z01 CM 06264-07 CL

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aims of this project are to 1) catalogue and identify the infections manifest both clinically and postmortem, 2) identify the predisposing factors which have contributed to the specific type of infection, 3) evaluate new techniques for more rapid and/or accurate diagnosis of infection.

Beginning July 1, 1970, each patient with suspected infection has been seen by one of the investigators who examines the patient and reviews the chart, both initially and throughout the course of the infection. Detailed records are kept of history, examination, laboratory data, etc. Similar details are obtained on infections detected at the time of postmortem examination.

A) Overall Clinical Infections - Review of all infections occurring among all patients treated for cancer by a small number of physicians under protocol conditions on a single hospital floor over a 6-year period has provided a unique opportunity to detect differences in infection type and incidence among different classes of tumor and to better understand the predisposing factors to each class of infections.

B) Pneumonia - The use of transtracheal aspiration and bronchial brushing techniques has resulted in a major increase in the premortem identification of the etiologic agents of pneumonia. The ability to promptly define the etiologic agent has allowed use of specific therapy at an early date with resultant improved response rates and diminished toxicity. (See Report #6913)

C) Torulopsis glabrata Infection - A retrospective analysis of microbiology records, autopsy information and clinical records disclosed that T. glabrata has caused infection in 27 patients during the past 48 months. A pulmonary portal of entry for this organism was suggested since 24/27 patients had primarily pulmonary involvement. Isolation of this organism from multiple sites on surveillance cultures was shown to correlate closely with subsequent clinical infection. T. glabrata infection is increasing in frequency and represents a significant contribution to infectious deaths among patients with advanced cancer.

D) Staphylococcus aureus - Surveillance of both clinical and microbiological reports indicated a rising incidence of S. aureus infection concomitant with an increased frequency of patient colonization with S. aureus. This recognition lead to evaluation of the potential routes of acquisition and an effective protocol approach to infection prevention (See #CM-06217, Infection Prevention).

E) Hodgkin's Disease - Infections that occurred in ninety-two previously untreated patients with Hodgkin's disease were reviewed from the time of laparotomy and splenectomy. Severe bacterial infections did not occur in any patient during initial therapy (radiation, drug therapy or both). Severe infection did occur in 8 profounded granulocytopenic patients with

recurrent neoplasm. Pneumococcal and Hemophilus sp infections were distinctly uncommon despite splenectomy. Herpes zoster occurred in 24% of patients but especially in those young females treated with radiation and adjuvant chemotherapy (64% incidence of zoster). Therefore, intensive therapy for initial disease does not predispose to severe infection. Splenectomy did not affect either the incidence or severity of infection during the period of observation of these adult patients.

Publications:

Schimpff, S.C., O'Connell, M.S., Greene, W.H., and Wiernik, P.H.: Infections in ninety-two splenectomized patients with Hodgkin's disease - a clinical review. Am. J. Med. 59: 695-701, 1975.

Schimpff, S.C.: Diagnosis of infection in patients with cancer. Europ. J. Cancer 11: 29-38, 1975.

O'Connell, M.J., Schimpff, S.C., Kirschner, R., Abt, A., and Wiernik, P.H.: Epithelioid granulomas in Hodgkin's disease. A favorable prognostic sign? JAMA 233: 886-889, 1975.

PROJECT NUMBER (DO NOT USE THIS SPACE)

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CM-06266-07 CL

PERIOD

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Therapy of Infection in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Senior Investigator	CL NCI
OTHER:	J. Aisner	Senior Investigator	CL NCI
	D.M. Hahn	Clinical Associate	CL NCI
	P.H. Wiernik	Chief, Section of Medical Oncology	CL NCI

COOPERATING UNITS (if any)

C.L. Fortner Patient Care Pharmacy
V.M. Young; N. Friedman Research Microbiology
H. Standiford Division of Infectious Diseases, University of Maryland
European Organization for Research on Treatment of Cancer, Antimicrobial Project Therapy Group

LAB/BRANCH

Clinical Branch

SECTION

Medicine

INSTITUTE AND LOCATION

NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.50

SUMMARY OF WORK (200 words or less - underline keywords)

Despite improvements in preventive techniques, infections remain as one of the most common serious complications of cancer therapy. The BCRC has continuing research programs designed to improve the therapeutic approach to these infections. The setting of new fever during granulocytopenia represents infection in ~70% of cases with bacteremia occurring in 20% thus necessitating the prompt empiric use of broad spectrum, bactericidal antibiotics. The BCRC has helped to form and participate in a multiinstitutional international antimicrobial therapy trial. Patients were randomly allocated to receive one of three antibiotic combinations as part of a prospective controlled trial which included 625 patient-trials (253 from the BCRC). Although each of the three regimens was efficacious for the majority of infections, carbenicillin plus gentamicin was found to be superior to cephalothin plus gentamicin for P. aeruginosa bacteremia, superior to carbenicillin plus cephalothin for E. coli, and equivalent to the other two regimens for Klebsiella and S. aureus bacteremia. In addition the cephalothin plus gentamicin combination produced significant renal dysfunction in 15% of patients compared to 2-3% for the other drug combinations. It was concluded that under the circumstances of this study, carbenicillin and gentamicin was the combination of choice.

Despite improvements in preventive techniques, infections remain as one of the most common serious complications of cancer therapy. The BCRC has continuing research programs designed to improve the therapeutic approach to these infections.

A) Empiric antibiotic therapy for the febrile granulocytopenic patient- The setting of new fever during granulocytopenia represents infection in 70% of cases with bacteremia occurring in 20% thus necessitating the prompt empiric use of broad spectrum, bactericidal antibiotics.

1) The BCRC has helped to form and participate in a multiinstitutional international antimicrobial therapy trial under the auspices of the European Organization for Research on Treatment of Cancer (EORTC). Patients were randomly allocated to receive one of three antibiotic combinations as part of a prospective controlled trial over the past two years. 625 patient-trials (253 from the BCRC) were conducted with approximately 50% of gram-negative bacillary bacteremias and 90% of all other infections responding to the initial antibiotic therapy alone. Carbenicillin plus gentamicin was found to be superior to cephalothin plus gentamicin for Pseudomonas aeruginosa bacteremia, superior to carbenicillin plus cephalothin for Escherichia coli, and equivalent to the other two regimens for Klebsiella and Staphylococcus aureus bacteremia. In addition, the cephalothin plus gentamicin combination produced significant renal dysfunction in 15% of patients compared to 2-3% for the other drug combinations. It was concluded that under the circumstances of this study, carbenicillin and gentamicin was the combination of choice.

2) Ticarcillin, a new semisynthetic penicillin similar to carbenicillin, was substituted for carbenicillin in approximately one half of the cases studied under the EORTC protocol at the BCRC. Ticarcillin in vitro is more active than carbenicillin, thus allowing its use at one half the carbenicillin dose. Results indicate that ticarcillin in combination with gentamicin or cephalothin was efficacious as carbenicillin and had the advantages because of lower dosage, of less sodium overload and possibly less hypokalemia. Ticarcillin will probably replace carbenicillin as the antibiotic of choice for P. aeruginosa.

3) Amikacin is a new aminoglycoside more active than gentamicin against many gram-negative bacilli, notably Pseudomonas aeruginosa. The combination of amikacin and cephalothin should, based on in vitro data, cover most of the bacterial organisms which produce infection in the compromised host and it is possible that, by giving lower doses more frequently, the potential for renal dysfunction can be avoided. To date, 25 granulocytopenic febrile patients have been given cephalothin plus amikacin as empiric antibiotic therapy at the BCRC. Renal dysfunction has not been a problem and efficacy, based on these few patients, appears excellent. This study will therefore be continued.

4) Serum bactericidal assays have been suggested as a means of pre-

dicting success or failure of antibiotic therapy in severe infection. Patients at the BCRC who were part of the EORTC trial had bactericidal assays performed against 10 strains each of Pseudomonas aeruginosa, K. pneumoniae, E. coli, and S. aureus on serum samples before and after antibiotic administration. The in vitro results paralleled the ultimate in vivo efficacy of the three antibiotic combinations, e.g., cephalothin plus gentamicin was inadequate for P. aeruginosa. This approach will therefore be continued with new antibiotic combinations such as amikacin plus cephalothin.

B) Fungal Infections - Infections with Candida sp, Torulopsis glabrata, Aspergillus sp and Phycomycete (Mucor) sp are increasing as more patients survive initial bacterial infections. Unfortunately, amphotericin B is the only antibiotic of defined efficacy yet the present response rate is low. Recently rifampin has been shown in vitro to be synergistic with amphotericin B against these yeast and fungal organisms. The BCRC has therefore begun a prospective randomized trial of amphotericin B versus amphotericin B plus rifampin as initial therapy for proven or strongly suspected fungal infection. Inadequate numbers of patients have been treated to date for meaningful evaluation. The study will continue.

Publications:

Gaya, H., Klastersky, J., Schimpff, S.C., Fiere, D., Widmaier, S., and Nagel, G.: Prospective randomly controlled trial of three antibiotic combinations for empirical therapy of suspected sepsis in neutropenic cancer patients. Europ. J. Cancer 11(Suppl.): 5-8, 1975.

Gaya, H., Klastersky, J. Schimpff, S.C., Tattersall, M.H.N.: Protocol for an international prospective trial of initial therapy regimens in neutropenic patients with malignant disease. Europ. J. Cancer 11(Suppl.): 1-4, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06275-07 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Cell Transfusion Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.A. Schiffer	Sr. Investigator	CL NCI
	J. Aisner	Sr. Investigator	CL NCI
	P.H. Wiernik	Head, Clinic. Br.	CL NCI
Other:	M. Schmuckler		CL NCI
	S. Hilbert		CL NCI

COOPERATING UNITS (if any)
Baltimore Red Cross, Community Blood and Plasma Service, Dr. Paul Terasaki, University of California, Los Angeles; Dr. R. Rubin, Johns Hopkins School of Public Health,Balto., Md. Balto Rh Typing Laboratory, Balto., Md.

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2	3

SUMMARY OF WORK (200 words or less - underline keywords)

The Cell Transfusion Program is designed to provide platelet and granulocyte transfusion for the therapy of patients with cancer. Studies in the past year have been used as a model, 30 blood processors have demonstrated that this is an efficient means of collecting platelet and granulocytes, and that the cells which are obtained are functionally and morphologically normal. Cryopreservation of platelets obtained using standard platelet pheresis techniques that frozen platelets can circulate and function hemostatically after transfusion. HL-A matched platelets have been frozen and stored and a large computerized donor file of HL-A matched donors has been created. Because of the problems of severe transfusion reactions appearing after the transfusions of granulocytes obtained by filtration of leukopheresis, studies are underway to improve collection and elution procedures. In addition, as part of the cell cryopreservation program, studies on the effects have various cryopreservatives (particularly dimetyl-sulfoxide) have been carried out. Clinical experience have demonstrated that granulocyte transfusion can be effective in the management of infections, and it has become our policy to use granulocyte transfusions earlier in the course of severe infections.

Project Description: Z01 CM 06275-07 BCRC

Aggressive therapy of patients with leukemia, lymphoma and other types of solid tumors has resulted in an increased need for blood cell components to treat the complications of pancytopenia. The purpose of the cell transfusion program is to provide platelets and granulocytes to treat and prevent hemorrhage and infectious complications in these patients.

1. Platelet Transfusion: Presently the bulk of platelets which are transfused are obtained from the Community Blood and Plasma Service and the Baltimore Red Cross. An increased number of platelets, many of which are HL-A matched for alloimmunized patients are now being obtained by multi-unit plateletpheresis done at the BCRC. To facilitate selection of unrelated donors, a computerized HL-A file of donors has been created which now contains the names of approximately 600 donors.

   In addition to plateletpheresis by the traditional serial centrifugation technique, a Model 30 Blood Processor (Haemonetics Corporation, Natick, Massachusetts) has been used extensively in the past year for platelet, granulocyte and lymphocyte procurement. As a result of a study which serial samples for blood counts were obtained in the out flow line of this apparatus, a standardized technique for efficient platelet and leukocyte collection has been designed. Approximately 85% of the platelets processed can be harvested using this technique and in vitro testing has demonstrated that these platelets are functionally normal. Electron micrographs also demonstrated that these platelets are morphologically normal while the results following transfusion have been quite satisfactory.

   HL-A matched platelets, pooled random donor platelets and autologous platelets obtained by plateletpheresis of patients with leukemia in remission have been frozen using 5% dimethyl sulfoxide as a cryoprotective agent and subsequently transfused after storage in the vapor phase of liquid nitrogen. The mean freeze-thaw loss was an acceptable 21% and the count increments achieved post-transfusion to thrombocytopenic patients were approximately two-thirds of that achieved with fresh platelets. The platelets appeared to be hemostatically acitive in that shortening of the bleeding time was noted and improvement of platelet aggregation was detected after in vivo circulation.

   Experiments relating to the effect of dimethyl sulfoxide on in vitro platelet function were also carried out. It was determined that DMSO had no permonent injurious effect on platelet size, aggregation, or morphology, or nucleotide and serotonin release. Transient alteration of aggregation and release was noted in the presence of DMSO but was eliminated after the platelets were washed.

2. Granulocyte Transfusion: As noted above, the mechanics of granulocyte collection using the Model 30 Blood Processor was also studied. When a rouleauxing agent is utilized, approximately 65% of the granulocytes can be harvested and satisfactory yields for transfusion can be obtained. In vitro functional studies have revealed that granulocytes and lymphocytes harvested by this procedure are morphologically and functional normal.

Further investigation into the problem of transfusion reactions occurring after transfusion of granulocytes obtained by filtration leucopheresis were carried out. In a series of paired transfusions using the same donor-recipient pairs and tow different methods of cell procurement, the rate of transfusion reactions using filtration leucopheresis cells was 55% whereas no transfusion reactions occurred using cells obtained by differential centrifugation. Efforts are currently underway to improve the filtration leucopheresis elution procedure so that less damage to the granulocytes occurs. Preliminary experiments indicate that it is difficult to elute granulocytes off nylon fibers by simple passage of various calcium chelating solutions over the fibers and that some sort of physical tapping (which is injurious to the cell) is required. The use of lidocaine, which can reverse monocyte adherence, as an aid to improve elution is presently being explored.

Our previous clinical impressions about the clinical efficacy of granulocyte transfusions have been further substantiated by our experience this year. It is now our policy to attempt to use granulocytes as soon as the diagnosis of severe infection is made.

All of the mechanical devices used to collect cells have many yards of plastic tubing. Studies will be carried out to determine the amount of plasticizer which is eluted and returned to normal donors during these procedures.

Publications:

1. Schiffer, C., Aisner, J. and Wiernik, P.: Transient neutropenia during continuous flow filtration leukopheresis. Blood 46: 17, 1975.

2. Buchholz, D., Schiffer, C., Wiernik, P., Betts, S., and Reilly, J.: Granulocyte transfusion: Donor response to repeated leukopheresis. In Goldman, J.M. and Lowenthal, R.M., (Eds.): Leucocytes: Separation, Collection and Transfusion. New York, Academic Press, 1975, pp. 177-191.

3. Buchholz, D., Schiffer, C., Wiernik, P. and Betts, S.: Granulocyte transfusion: A low cost method for filtration leukopheresis. In Goldman, J.M. and Lowenthal, R.M. (Eds.): Leucocytes: Separation, Collection, and Transfusion. New York, Academic Press, 1975, pp. 137-145.

4. Sanel, F., Aisner, J., Tillman, C., Schiffer, C. and Wiernik, P.: Evaluation of granulocytes harvested by filtration leukopheresis: Functional, histochemical and ultrastructural studies. In Goldman, J.M. and Lowenthal, R.M. (Eds.): Leucocytes: Separation, Collection and Transfusion. New York, Academic Press, 1975, pp. 236-251.

5. Schiffer, C.A., Whitaker, C.L., Schmukler, M., Aisner, J. and Hilbert,S.L.: The effect of dimethyl sulfoxide on in vitro platelet function. Transfusion 15: 511, 1975.

6. Schiffer, C.A., Buchholz, D.H., Aisner, J., Wolff, J.H. and Wiernik, P.H.: Frozen autologous platelets in the supportive care of patients with leukemia. Transfusion, 1976 (in press).

7. Schiffer, C.A., Aisner, J., and Wiernik, P.H.: Current approach to granulocyte transfusion and cell component collection. Proc. of the Internat'l Advance Component Seminar, Haemonetics Research Institute, 1976 (in press).

8. Aisner, J., Schiffer, C.A., Wolff, J.H., and Wiernik, P.H.: A standardized technique for efficient platelet and leukocyte collection using the Model 30 Blood Processor. Transfusion, 1976, (in press).

9. Rubin, R. and Schiffer, C.A.: Fate in humans of the plasticizer di(2-ethylhexyl) phthalate, arising from transfusion of platelets stored in vinyl plastic bags. Transfusion, 1976, (in press).

PROJECT ... (DO NOT USE THIS SPACE)

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06276-03 CL

PERIOD ...
July 1, 1975 through June 30, 1976

TITLE ... (30 characters or less)
The Chemotherapy of Metastatic Testicular Carcinoma

NAMES, ... AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Head, Section of Medical Oncology	CL NCI
Other:	A. Collier Smyth, M.D.	Clinical Associate	CL NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY ... (300 words or less - underline keywords)

The addition of bleomycin to 5-fluorouracil, cyclophosphamide, methotrexate, and vincristine has been shown, in preliminary evaluations of this study, to markedly enhance the efficacy of this combination of agents against metastatic testicular carcinoma. In addition, useful activity of adriamycin plus cis-diamine-dichloroplatinum has been identified in this study.

PHS-6040
(12-75)

Project Description:

Objectives are to assess the efficacy of various anti-tumor chemotherapeutic agents in metastatic testicular carcinoma. Patients are currently randomized between a combination of drugs consisting of fluorouracil, cyclophosphamide, methotrexate, and vincristine and a combination including the same drugs plus bleomycin given 12 hours after the dose of vincristine. Patients failing this protocol receive adriamycin plus cis-diamine-dichloroplatinum.

Twenty-nine patients have currently been entered into the study. Objective tumor regression has been observed in 20 patients. Both regimens are generally well tolerated and no major life threatening toxicity has been encountered. It is too early to determine whether the addition of bleomycin to the other agents will result in a higher remission rate or longer remission duration.

Testicular carcinomas account for a significant proportion of tumors in young male adults. Metastatic testicular carcinoma has been shown to be responsive to several different classes of anti-tumor agents by this group and others. The present study shows promise of achieving results superior to those previously reported.

Publications:

1. Stechmiller, B.K., Wiernik, P.H., Shin, M., Satterfield, J.: Maturation of Metastatic Charatocarcinoma Following Chemotherapy to a Mass Pathologically Indistinguishable from a Mediastinal Enteric Cyst. Chest (in press), 1976.

SMIT [illegible] PROJECT NUMBER (Do NOT use this space)

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06277-05 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Chemotherapy of Adult Acute Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF [illegible] AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Branch	CL	NCI
Others:	Joseph Aisner, M.D.	Senior Investigator	CL	NCI
	A. Collier Smyth, M.D.	Senior Clinical Assoc.	CL	NCI
	J. Leonard Lichtenfeld, MD	Senior Investigator	CL	NCI
	Bruce Chabner, M.D.			

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	2	3

SUMMARY OF WORK (200 words or less - underline keywords)

A seven day continuous intravenous infusion of cytosine arabinoside in association with a three day schedule of intermittent daily doses of daunorubicin has been shown to be a highly effective chemotherapeutic regimen for the induction of remission in adults with acute nonlymphocytic leukemia. A complete remission rate of 70% is being obtained with this combination of agents. Other newer agents such as the epipodophyllotoxin derivative of EP 16-213 and neocarcinostatin are under investigation for their antileukemic activity and early indications are that these drugs have activity against this disease.

Reverse transcriptase has been identified in the bone marrow cells of leukemia patients prior to treatment and in morphologically normal peripheral cells during complete remission. One interpretation of this data is that morphologically normal and functionally normal granulocytes in the peripheral blood of leukemia patients in complete remission are derived from leukemic cells.

PHS-6040
(12-75)

I. Effect of Single Agents

A. VP16-213

This epipodophyllotoxin derivative has been reported by Mathe to be active in acute nonlymphocytic leukemia. We have studied this drug as a single agent in previously treated or relapsed patients and found a very low response rate in such patients. In combination with azacytidine, little addition activity was identified. A total of 30 patients have been treated.

B. Neocarcinostatin

Phase I-II trials with this Japanese drug have been started in acute leukemia patients no longer responsive to more conventional therapy. Four patients have been treated thus far and results are not yet available.

II. Effect of Multiple Drugs in Combination

A. Daunorubicin and cytosine arabinoside

In a study combining a 3 day schedule of daunorubicin and a 7day schedule of cytosine arabinoside a 70% complete remission rate was obtained in previously untreated patients with acute nonlymphocytic leukemia irrespective of age. Biochemical testing of leukemic bone marrow cells did not allow for selection of favorable groups of patients to be treated with cytosine arabinoside (kinase-deaminase ration) or daunorubicin alone (daunorubicin reductase level).

B. Vincristine, Thioguanine, Pyrimethamine and Dexamethasone vs. Vincristine, Thioguanine, Methotrexate and Prednisone for Remission Induction in Adults with Acute Lymphocytic Leukemia:

This program is designed to test whether or not pyrimethamine administered initially to adults with acute lymphocytic leukemia can prevent or delay the onset of meningeal leukemia in long-term survivors.

Additionally, the protocol was designed to test whether or not dexamethasone, a steroid that mobilizes granulocytes might be associated with fewer infections during induction than prednisone, a steroid that inhibits granulocyte mobilization. A complete remission was obtained in 53% of patients in the median duration of survival of remitting patients is in excess of 18 months. Approximately 40% of patients on the study have developed meningeal leukemia.

III. Maintenance Therapy for Remission in Adults with Acute Non-Lymphocytic Leukemia.

A. High dose methotrexate with citrovorum rescue.

Intensive courses of high dose methotrexate with citrovorum rescue are being utilized, along with other drugs, for maintenance offirst complete remission. Although some relatively long term remissions have been achieved it is too early to completely evaluate this regimen.

B. Cyclophosphamide and BCNU.

This regimen has been reported to be highly efficacious for patients in their first remission. We are testing the regimen in patients who have attained a second remission after relapse. Preliminary data suggest that the regimen is well tolerated.

IV. Biochemical Indicators of Disease Activity in Acute Non-Lymphocytic Leukemia.

A. Serum and Urine Muramidase Studies

We continue to confirm the utility of serum muramidase levels in differentiating

between acute monocytic, myelomonocytic, and myelocytic leukemia. In addition, we continue to observe that serum muramidase levels accurately reflect disease activity in a large proportion of patients with acute non-lymphocytic leukemia. It has also become evident that patients with initially elevated serum muramidase levels who achieve complete remission and normal serum muramidase levels have a longer remission than those who achieve hematologic and clinical remission but maintain elevated serum muramidase levels.

B. Carcinoembryonic Antigen:
We have observed that approximately 30% of adult acute non-Lymphocytic leukemia or acute lymphocytic leukemia have abnormal levels of carcinoembryonic antigen in their serum. Unfortunately, these levels do not correlate well with disease activity. Most patients undergoing therapy have a rise for weeks or months in their plasma CEA whether or not they respond to therapy. In addition, when patients relapse, the presence or absence of CEA in their plasma at that time does not correlate with findings at the time of presentation.

C. Serum Immunoglobulin Concentrations in Patients with Acute non-lymphocytic Leukemia:
We have measured quantitatively serum IgA, IgD, IgE, and IgM in patients with acute non-lymphocytic leukemia prior to therapy, during therapy, during remission and relapse. Most patients with acute non-lymphocytic leukemia present with decreased levels of IgG in their serum. When complete remission is achieved, these levels become normal.

D. Reverse Transcriptase in Leukemic Cells:
This enzyme of presumed viral origin is being looked for in cells and plasma from our leukemia patients by Dr. Sol Spiegelman, New York. Results are preliminary at this point. 50 patients have been studied prior to therapy and the enzyme was present in the white cells of each. In addition, most patients in complete remission continue to have the enzyme detectable in their white cells. We are now looking for the enzyme in the various fractions of the buffy coat of patients in remission, and in autopsy specimens.

E. Cell-Mediated Immunity in Acute Non-Lymphocytic Leukemia:
Cell-Mediated immunity in acute non-lymphocytic leukemia, either at initial presentation or in subsequent relapse,was determined among 98 patients by recall-antigen skin testing and in 67 patients by attempted sensitization and challenge with dinitrochlorobenzene (DNCB). The leukemic patient's subsequent prognosis, in terms of attainment of complete remission of his leukemia, duration of remission, and survival could not be correlated with the pretherapy demonstration of intact delayed cutaneous hypersensitivity.

Summary of Major Findings:
1. Daunorubicin and cytosine arabinoside given in intensive courses produce a complete remission rate superior to that of other regimens tested at this institution or elsewhere.
2. Serum muramidase levels are useful in following disease activity in adult acute

non-lymphocytic leukemia.

3. Carcinoembryonic antigen is present in increased amounts in the minority of patients with acute non-lymphocytic leukemia. However, serial determinations are useless in following disease activity.

4. Cell-mediated immunity in leukemia patients prior to therapy may not be important in determining response.

5. The presence of reverse transcriptase in the white cells of leukemia patients in remission may indicate the necessity for further induction therapy. It may also suggest that "normal" granulocytes present during remission represent matured leukemic cells.

These studies indicate continued improvement in our ability to arrest disease activity in adults with acute non-lymphocytic leukemia in an ever increasing number of patients. In addition, biochemical parameters are being identified that reflect accurately disease activity in many patients with acute non-lymphocytic leukemia. The identification of such factors may lead to more rational therapy in the future. Demonstration of reverse transcriptase in leukemic cells may have etiologic significance.

PROJECT NUMBER (DO NOT USE THIS SPACE)

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 CM 06280-04 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Combination Chemotherapy for Advanced Hodgkin's Disease.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	:	P.H. Wiernik	Head, Clin. Br.	CL NCI
		C.H. Diggs	Sr. Clin. Assoc.	CL NCI
Other	:	J.A. Levi	Sr. Clin. Assoc.	CL NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

Fifty patients with advanced Hodgkin's Disease were treated with a combination of cyclophosphamide, vinblastine, procarbazine, and prednisone (CVPP) in every three week regimen. Patients who achieved complete remission were randomized to receive maintenance therapy consisting of monthly alternating CCNU and vinblastine or to be followed on no therapy. Thirty-one patients (62%) achieved complete remission with the fewest remissions being seen (3/8) in those patients who had previously both chemotherapy and radiation therapy. Maintenance therapy to date has no significantly prolonged remission duration or survival. Patients who received more than six courses of induction therapy have had longer remissions and fewer relapses than those receiving only six courses regardless of maintenance therapy status. CVPP is an effective regimen at inducing remissions in advanced Hodgkin's disease. Maintenance therapy has not yet been shown to be beneficial.

PHS-6040
(12-75)

The objectives are to teach the theory and practice of medical oncology to medical students and house officers; to acquaint such individuals with newer aspects of chemotherapy, radiotherapy, and surgery as they apply to neoplastic disease; and to provide a consultative service for difficult patients with neoplastic diseases for other physicians in the Baltimore area.

During their second year, medical students from the University of Maryland are assigned to this service on a regular basis for their physical diagnosis class. Students from the University of Maryland and Johns Hopkins are assigned here during elective periods for a clinical clerkship in medical oncology. In addition, house officers from the Greater Baltimore Medical Center and The University of Missouri at Kansas City School of Medicine have been assigned here for elective periods of their house officer year. University of Maryland Assistant Residents in Medicine rotate through the Section of Medical Oncology on a regular basis. Physicians in the community refer patients here for out-patient consultation. If the patient meets the qualifications for one of our protocol studies, he is accepted in transfer and admitted to BCRC. If not, the patient is completely evaluated as an out-patient and returned to his physician with specific recommendations for therapy.

Students and house officers who have rotated through the BCRC are recognizing that medical oncology is a bona fide subspecialty of internal medicine. They have learned that patients with neoplastic diseases require special care based on special knowledge. It has been suggested by some local private physicians that the general care of patients with neoplastic diseases in the community has advanced because of our consultation service.

The dissemination of information concerning the latest techniques in the diagnosis and therapy of cancer patients is of paramount importance in advancing the attack on this disease. By training potential physicians at this time, early during their student and house officer years, such information will be brought to the community in a more orderly and useful way than is sometimes possible. By seeing patients in consultation, we have the opportunity to educate the patient's physician as to modern techniques of management for a given neoplastic disease. Both of these teaching functions we engage in are of significant import in elevating the level of care available to patients with cancer in this area.

[illegible] NOT

PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 CM 06[illegible] 04 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Census: A Remote Access Patient Information Storage and Retrieval System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	William L. Robinson			
	Robert J. Esterhay, M.D.	Senior Investigator	CL	NCI
Others:	Paul Chang, M.D.	Clinical Investigator	CL	NCI

COOPERATING UNITS (if any)
Section of Neurological Surgery, NCI, BCRC: Nursing Service, NCI, BCRC,: Section of Microbiology, NCI, BCRC: Clinical Research Pharmacy, NCI, BCRC: Administrative Officer, NCI, BCRC: Section of Medical Oncology, NCI, BCRC.

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	4	0

SUMMARY OF WORK (200 words or less - underline keywords)

The CENSUS system is a set of computer programs which enables the storage and retrieval of a data base of information on cancer patients treated at the Baltimore Cancer Research Center. The computer programs and the data base are accessed from remote terminals connected to a commercial time-sharing computer facility. Structured data input programs facilitate the addition of new information to the data base. A flexible retrieval program produces patient listings sorted by patient name, cancer diagnosis, attending physician, status, and/or research protocols. A medical secretary updates the data base daily and then retrieves a CENSUS report which lists all inpatients currently on the Medical Oncology outpatient service and all Medical Oncology clinic visits made that day. A general purpose information management software library performs data base manipulations and retrievals which previously required the development of special purpose computer programs. Several programs have been written to produce administrative and accounting tabulations of the data stored on the system. The system has been in operation since August of 1971 and the data base currently contains records for over 1000 cancer patients.

PHS-6040
(12-75)

Objectives:

1. To compile, update, and retrieve from a data base of medically relevant information on cancer patients that is centralized, readily accessible, and maintained by a single non-technical individual.
2. To generate a daily report on all in-patients and their locations, along with their vital statistics and current therapy data, to be distributed to all involved in the tasks of clinical research, patient care and administration.
3. To develop flexible data retrieval programs which generate a variety of useful patient summaries keyed on any of several patient parameters.

Methods Employed:

1. Information is compiled and recorded daily in a data input log by a medical secretary.
2. A computer program accepts the input datum from the secretary, checks for logical content and accuracy, and records it on the data base.
3. A second computer program generates a Daily Census Report which reflects the updated status of the data base.
4. The report is then distributed to the scientific and professional staff, remote contracting laboratories, and administrative office.
5. The programs and data base are executed and stored on a commercial timesharing service.
6. Mass memory disk files and remote terminals form the storage and access mediums.

The daily census report has been utilized by all sections and services of the Cancer Center.

1. Admissions Office: used to assign new patients to their physicians and to distribute the patient load evenly among the physicians.
2. Clinical Associates: used at evening sign-out rounds as a checklist of patients with speical problems that may require extra care overnight.
3. Sections of Medical Oncology and Neurological Surgery. The master retrieval programs are most useful to the physician performing a chart review to evaluate treatment protocols and who requires listings of the patient population which are sorted by status, cancer diagnosis, research protocol, etc.
4. Clinical Research Pharmacy Service: used to fill the unit drug cart patient trays.
5. Section of Microbiology: provides the laboratory staff and outside contracting laboratories with relevant information on patients whose cultures are being analyzed. The permanent records of all beds a patient has occupied provide a useful epidemiological tool in detecting the spread of infectious organisms. The data base is also accessed by computerized microbiology reporting systems at the BCRC (see Project Report Z01 CM 06039-04 BCRC).
6. A general-purpose information management software library now performs data base manipulations and retrievals which previously required the development of special purpose computer programs.
7. Several programs have been written to produce administrative and accounting tabulations of the data stored on the system.

The content and flexibility of the retrieval summaries generated by the Census System have provided the Research Center with a valuable "index" to patient records. The daily routine of update and audit have eliminated the inaccuracies and incompleteness of previous disjoint and ill-kept data bases. Four years of continuous operation of the Census System have provided BCRC with a substantial data base of information on cancer patients. Future developments will concentrate on effective utilization of the information thus obtained. These developments will be in the form of programs to correlate census data with data compiled by other data systems at the BCRC.

Because of these findings and the known potential for transient hepatic dysfunction with streptozotocin therapy bromsulphalein (BSP) dye retention studies were also performed in two patients receiving adriamycin and streptozotocin both before and 24 hours after initiation of treatment. In both patients, only one of whom had clinically evident (transiently elevated SGOT and bilirubin) hepatic dysfunction, the BSP dye retentions changed from normal to abnormal 24 hours after treatment was initiated. It was thus suggested that there is a pharmacokinetic drug-drug interaction between the two agents and that the probable gross pathogenetic mechanism involved clinical and possibly sub-clinical hepatic dysfunction induced by streptozotocin, slowing the elimination of adriamycin via its major elimination pathway.

Parallel pharmacokinetic studies were also performed in the same patients, utilizing a recently developed radio-immunoassay which measures both parent adriamycin, adriamycin aglycone (whose activity is unknown). Kinetic analysis of the radio-immunoassay generated plasma clearance curve revealed no significant difference in the excretory half-life. As in the fluorescence assay of the plasma disappearance curve, however, clearance of adriamycin, a sensitive measure of its hepatic elimination, was also significantly decreased compared with curves generated for patients receiving adriamycin alone. The differences in clearance of adriamycin measured by both assays confirmed a definite impairment of excretion of active adriamycin when given in combination with streptozotocin. The additional slowing of the terminal excretory half-life of adriamycin fluorescence might additionally suggest the presence of additional fluorescent metabolites, not measured by the radio-immunoassay, which are present for prolonged periods of time in the combination treated patients. Whether these are actually toxic metabolites, responsible for the increased myelosuppression and mucositis seen in these patients is unknown. It is of interest to note, however, that increased myelosuppression and mucositis without an increase in therapeutic effect is the same clinical consequence seen when patients with intrinsic liver disease are given adriamycin in full doses.

This clinical study has now been terminated and submitted for publication. Previously untreated patients with advanced sarcomas are now being treated according to Part C below. Further pharmacokinetic studies of the specific adriamycin metabolites are now being performed to attempt to further elucidate the pathogenesis of this drug-drug interaction.

B) Intravenous Methyl CCNU for Advanced Sarcomas - This study has been terminated and recently published.

C) Adriamycin + Actinomycin-D for Advanced Sarcomas - As indicated in A above, adriamycin has now become the cornerstone of therapy of inoperable and metastatic sarcomas, having been identified as the most effective single drug in this group of tumors. It has, however, significant dose limiting toxicity in that 10-30% of patients who receive more than 550mg/M^2 (cumulative dose) later suffer irreversible cardiac failure.

Antinomycin-D an S phase, cell cycle-specific, antineoplastic antibiotic is known to be an effective palliative drug in approximately 20-30% of patients with advanced sarcomas. It is hoped that by combining these two agents in a cytokinetically-rational fashion, it may be possible to increase the rate and duration of response of patients with advanced sarcomas to chemotherapy without an undue increase in mucositis and myelosuppression which are common to both agents.

At present, this study has just been initiated and insufficient data is available for discussion.

Publications:

Chang, P., Riggs, C., Reich, S., and Bachur, N.R.: Aggravation of adriamycin toxicity by streptozotocin: Clinical-pharmacologic correlation. Proceedings of the American Society of Clinical Oncology, 1976.

Chang, P., Riggs, C., Wiernik, P., and Bachur, N.: Clinical-pharmacologic correlation of augmented adriamycin toxicity caused by streptozotocin. 11th Joint Meeting of the Professional Associates of the U.S.P.H.S., May, 1976.

Chang, P., Reich, S., Riggs, C,. Schein, P., Bachur, N., and Wiernik, P.: Adriamycin and streptozotocin therapy of advanced sarcomas. Proceedings of the American Association of Cancer Research 16: 82, 1975.

Chang, P.: Phase II evaluation of intravenous methyl CCNU in sarcomas. Proceedings of the American Society of Clinical Oncology, 16: 236, 1975.

Chang P., Levine, M.A., Wiernik, P.H., and Walker, M.D.: A Phase II Study of Intravenouely Administered Methyl CCNU in the Treatment of Advanced Sarcomas. Cancer 37:615-619, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06907-03 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Colony Stimulating Factor and Colony Forming Capacity from Patients with Neoplasms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David Van Echo, M.D.	Clinical Associate	CL NCI
Other:	Peter H. Wiernik, M.D.	Head, Section of Med. Oncology	CL NCI

COOPERATING UNITS (if any)

Medical Service, BCRC, NCI

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

SUMMARY OF WORK (200 words or less - underline keywords)

Ithas been determined in this study that colony forming units occur less often in the bone marrow of patients with acute leukemia than in patients with no hematologic disease.

Project Description:

Since the 1960's, numerous reports of the successful in vitro growth of human peripheral blood and bone marrow have been published. In small groups of leukemia patients, CSF and CFC have been shown to rise during remission and fall during relapse. In addition, leukemic cells have been shown to mature in vitro on occasion. The large leukemic population of the Baltimore Cancer Research Center would allow more adequate assessment of these findings. Additionally, the mechanism of delayed cumulative hematologic suppression with nitrosourea therapy has not been explained. The in vitro culture technique would allow us to determine if decreased CSF (stimulatory) or decreased CFC (target) is the basic deficit caused by nitrosourea chemotherapy. The large malignant central nervous system neoplasm patient population of the BCRC would allow a unique opportunity to study this peculiarity of the nitrosoureas. Primary central nervous system neoplasm patients rarely have metastases to bone marrow producing areas. The following assessment will be made:

1. Measurement of CSF and CFC in various stages of hematologic malignancy.
2. Measurement of CSF and CFC in various stages of solid tumors infiltrating bone marrow.
3. Measurement of CSF and CFC in patients in various stages of chemotherapy, particularly with nitrosourea compounds..
4. Morphologic studies of in vitro grown leukemic cells.

The growth technique involves incubation for a short period of time a source of CSF such as urine, peripheral white blood cells, plasma or serum in McCoy's media, fetal calf serum, and dilute agar or methyl cellulose. Bone marrow from human beings or mice are evenly dispersed in the same semisolid culture medicum and incubated over a prepared feeder layers. The cultures are incubated in 35 mm petri dishes for 10-21 days in a humidified CO_2 incubator at 37° C, removed and examined under a dissecting microscope. Culture dishes are examined for colony counts and cell morphology.

The project is in its early stages. More consistency in the growth of cells is required at this time. In vitro studies using this technique will help in the understanding of the mechanisms of hematologic malignancies. Additionally, it is hoped to develop better understanding of the unusual delayed, cumulative toxicity of nitrosoureas. Further refinements in the culture techniques are anticipated. When consistent growth is obtained, detailed studies of hematopoietic function will be under taken. We also hope to develop some information regarding tumor burden levels in the bone marrow.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM06913-03 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Bronchial Brushing for Pulmonary Infiltrates in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Aisner, M.D.	Senior Investigator	CL NCI
Others:	Stephen C. Schimpff, M.D.	Senior Investigator	CL NCI
	Viola M. Young, Ph.D.	Head, Microbiology	CL NCI
	Peter H. Wiernik, M.D.	Head, Section of Medical Oncology	CL NCI

COOPERATING UNITS (if any)
Research Microbiology, BCRC, Pathology and Radiology Departments, University of Maryland Hospital

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1/2	1/2	0

SUMMARY OF WORK (200 words or less - underline keywords)
Since the immunologically compromised patient frequently develops pneumonias which are difficult to diagnose, aggressive diagnostic techniques are warranted. This study established a method of diagnosing unexplained pulmonary infiltrates utilizing selective bronchial brushing per oral fiberoptic bronchoscopy, or percutaneous lung biopsies when other methods fail. Forty patients with diffuse nodular infiltrates have undergone such procedures, of twelve patients not considered clinically infected, eight proved to have pulmonary metastases, three proved to have interstitial fibrosis, and one proved to have a collapsed lobe. Twenty-eight patients were clinically presumed infected and seventeen were proven to have pulmonary infection. Fourteen of the seventeen were etiologically documented by these procedures. Eleven of the twenty-seven patients had no etiology established by any method, although eight of these eleven patients were receiving broad spectrum antibiotics at the time of the procedure. The diagnoses included a variety of micro-organisms, both bacterial and fungal. Significant, but non fatal complications occurred and were acceptable risks for the patient population. There was no mortality associated with the procedures and despite pancytopenia, bleeding was not a problem.

Project Description: Z01 CM 06913 03 CL

The immunologically compromised and myelosuppressed cancer patient frequently develops pulmonary complications, especially infections. Such pneumonias are a contributing factor in the demise of many of these patients. A major reason for the high mortality lies in difficulty in establishing an etiological agent and thereby, providing appropriate antimicrobial therapy. Since there is an established low yield of noninvasive culture techniques for pneumonia occurring in such patients, more aggressive diagnostic techniques are warranted. Myelosuppressed patients frequently carry gram-negative organisms and fungi in their nasopharynx which will contaminate expectorated sputum. The purpose of this study is to establish a method for diagnosing unexplained pulmonary infiltrates where conventional methods (sputum induction and transtracheal aspiration) have been unsuccessful. This study utilized a transtracheal approach with selective bronchial brushing peroral fiberoptic bronchoscopy, and percutaneous lung biopsies when other methods had failed.

As of February 1976, forty patients with diffuse or nodular pulmonary infiltrates have undergone transtracheal bronchial brushing. Conventional methods including transtracheal aspiration, failed to establish a diagnosis in all cases. Twelve patients were not considered clinically infected. Eight proved to have pulmonary metastases of which only one was diagnosed by this technique. Four patients for whom a diagnosis was obtained by brushing subsequently proved to have interstitial fibrosis (Hamman-Rich disease) (1). Twenty-eight patients were clinically presumed infected. Ultimately, seventeen of these twenty-eight patients were proven to have pulmonary infection, and fourteen of the seventeen were etiologically documented by brushing. For eleven of the twenty-seven presumed infected patients, no etiology could be established by any method. Eight of these eleven patients were receiving broad spectrum antibiotics at the time of the procedure. Diagnosis established by the brushing procedure included Aspergillus pneumonia, Phycomycetes pneumonia, Torulopsis glabrata pneumonia, staphylococcal pneumonia, streptococcal pneumonia, Mycobacterium tuberculosis, anaerobic bacterial pneumonia, and metastatic sarcoma. Significant but non-fatal complications including hemoptysis, pneumothorax and cervical cellulitis occurred in seven patients. There was no mortality associated with the procedure. Despite pancytopenia, bleeding was not a problem.

Proposed Course:

Continue investigation.

Publications:

1. Sickles, EA, Young, V.M., Greene W.H., Wiernik, P.H.: Pneumonia in Acute Leukemia. Ann Intern Med 79: 528-534, 1973.
2. Aisner, J., Schimpff, S.C., Bennett, J.E., Young, V.M., Wiernik, P.H.: Aspergillus Infections in Patients with Cancer: An Association with Fire-Proofing Materials in a New Hospital. JAMA 235: 411-412, 1976.
3. Aisner, J., Kvols, L.K., Sickles, E.A., Schimpff, S.C., Wiernik, P.H.: Transtracheal Selective Bronchial Brushing for Pulmonary Infiltrates in Patients with Cancer. Chest 69: 367-371, 1976.
4. Aisner, J., Schimpff, S.C., Sutherland, J.C., Young, V.M., Wiernik, P.H.: Torulopsis Glabrata Infections in Patients with Cancer. Increasing Importance and Relationship to Colonization. Am J of Med (in press).
5. Aisner, J., Schimpff, S.C., Bennett, J.E., Sutherland, J.C., Young, V.,M., Wiernik,

P.H.: Increased Incidence of Aspergillus Infections in Cancer Patients: An Association with Fire-Proofing Materials. Clin Res 23: 300a, 1975.

6. Aisner, J., Schimpff, S.C., Hahn, D.M., Wiernik, P.H.: Fungal infections in patients with Cancer: Changing Frequency Associated with Broad Spectrum Antibiotics. Clin Res, 23: 586a, 1975.

7. Aisner, J., Schimpff, S.C., Sutherland, J.C., Young, V.M., Wiernik, P.H.: Torulopsis Glabrata infections in patients with cancer: An Increasing Incidence. Clin Res 23: 300a, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (DO NOT USE THIS SPACE)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06914-03 CL

PERIOD COVERED
July 1, 1975, through June 30, 1976

TITLE OF PROJECT (50 characters or less)
Radiotherapy for Localized Inflammatory Lesions in Patients with Acute Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John A. Levi, M.D.	Clinical Associate	CL NCI
Others:	Stephen C. Schimpff, M.D.	Senior Investigator	CL NCI
	Peter H. Wiernik, M.D.	Head, Clinical Branch	CL NCI

COOPERATING UNITS (if any)
Section of Radiotherapy, University of Maryland Hospital, Baltimore, Maryland

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to determine the value of superficial radiation therapy to patients with localized infected lesions with acute leukemia and associated neutropenia versus supportive care alone. Patients who have a local infection of the perirectal region, axilla, or any other subcutaneous tissue, are randomized to receive either supportive care alone or supportive care plus 400 rads of localized radiation which was given in a double blind fashion. Thirty patients have been entered into this study to date. Sixteen patients have received local radiation therapy and fifteen lesions have completely regressed by day 11 and one patient had progressive lesions. Fourteen patients did not receive radiation therapy and eleven had complete resolution by day 16, one partial resolution after 3 weeks and three lesions progressed despite local care. One patient in the non-irradiated group has suffered from disseminated infection as a result of the local lesions. There have been 9 recurrences of these local infections among the thirty patients and three of the recurrences have been in sites previously irradiated, while six recurred in sites not previously irradiated. It would appear that localized radiation therapy has beneficial effects on the rate of resolution and prevention of recurrences, but it is not apparent that this procedure has been of any particular value in preventing disseminated infection.

PHS-6040

Project Description: Z01 CM 06914-03 CL

To define the optimal management of these localized lesions in patients with acute leukemia and in particular to determine in a controlled fashion whether low-dose irradiation therapy administered to sites of local inflammation and infection will facilitate the healing process and prevent the development of disseminated infection with its associated morbidity and mortality.

Localized irradiation therapy has been utilized in the past in uncontrolled studies in patients with leukemia and results achieved have suggested some benefits to be derived from its use.

Patients with acute leukemia who develop a localized superficial inflammatory lesion which is not secondary to a previously proven disseminated infection are randomized to receive local care alone to these lesions or local care plus 400 rads of megavoltage irradiation directed to the local lesion and given over a 2 day period. A further 400 rads is given in 7 days irrespective of immediate results. Patients are separated into three groups: (a) perianal or perineal lesions, (b) axillary lesions, (c) other sites. All patients go down to the Radiotherapy Department and allocations to radiotherapy are not communicated to the clinical staff until the patients trial is ended.

Thirty patients have been entered into this study, 16 patients have received local radiotherapy and 15 lesions completely regressed by day 11 and 1 patient had progressive lesions, 14 patients did not receive radiotherapy and 11 had complete resolution of lesions by day 16, one partial resolution after 3 weeks and 3 lesions progressed despite local care. One patient in the non-irradiated group has suffered from disseminated infection as a result of the local lesions. There have been nine recurrences of these local lesions among a total of 30 patients, and three of these recurrences occured in sites previously irradiated while six recurred in sites that were not previously irradiated. Although it is not possible as yet to come to any meaningful conclusions, it would appear that localized radiotherapy has had some beneficial effects on the rate of resolution and prevention of recurrences. However, it is not apparent that the addition of irradiation to local therapy has been of any particular value in preventing disseminated infection.

Overwhelming infection remains the principal cause of death in patients with acute leukemia and further methods to prevent and treat such infections are required. Patients with acute leukemia have reduced body defenses to cope with local infections and a significant mortality has been associated with dissemination from these sites in the past. The early results reported here show some promise for the use of local radiotherapy in speeding the healing of such lesions and preventing recurrences. However, all forms of supportive care appear able to prevent disseminated infection.

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
PUBLIC HEALTH SERVICE
PROJECT NUMBER (DO NOT use this space)

NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06915 03 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (75 characters or less)

Staging Laparotomy and Combined Modality Therapy in Early Hoogkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John A. Levi, M.D.	Clinical Associate	CL NCI
	Peter H. Wiernik, M.D.	Head, Section of Medical Oncology	CL NCI
Other:	Robert A. Slawson, M.D.	Acting Director, Div. Radiation Therapy	Univ. Md. Hospital

COOPERATING UNITS (if any)

Radiotherapy Service and Surgery Service, University of Maryland Hospital, Baltimore, Maryland

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

SUMMARY OF WORK (200 words or less - underline keywords)

The aims of this study were to determine the value of staging laparotomy in Stage I and II Hodgkin's Disease and further optimal initial therapy following laparotomy. Following the completion of initial clinical evaluation, patients were randomized to receive laparotomy or no laparotomy. Those patients receiving no laparotomy received extended field radiation alone and to date, 4 patients have been entered and all have achieved complete remission and there have been no relapses to the present time (range 3 to 19 months). 23 patients with pathologic stage I to IIA disease were randomized following laparotomy to one of the other 3 treatment arms and 4 patients have received limited field radiation alone, 11 patients extended radiation alone and 8 patients involved field radiation plus MOPP. There have been 6 relapses, 2 among the patients receiving involved field radiation alone, and 4 among the patients receiving extended field radiation alone. There have been no relapses among the involved field radiation plus MOPP. Patients with E stage disease of the lung comprised 3 of the 6 relapses and it has been decided that for the remainder of the study, patients with E stage disease will be removed from this protocol. It is too early at this time to make any specific assessments, although it does appear that patients receiving irradiation plus chemotherapy are continuing to show improved results over those receiving irradiation alone. It is too early to make any specific statements regarding the patients who received extended field irradiation alone without prior laparotomy.

Project Description: Z01 CM 06915-03 CL

1. To determine whether modification of radiation therapy ports on the basis of staging laparotomy findings will significantly improve survival in patients with clinically localized Hodgkin's disease, as compared to radiation therapy administered without staging laparotomy.

2. To determine whether the addition of adjuvant chemotherapy to limited field radiation therapy will result in improved survival compared to limited field radiation alone or extended field radiation alone or extended field radiation alone in patients with clinically localized disease who have undergone staging laparotomy.

Patients with newly diagnosed Hodgkin's disease undergo complete clinical evaluation including history, physical examination, serum chemistries, and lymphangiography. At this point patients are randomized to receive extended field radiation therapy alone, or alternatively to undergo staging laparotomy with subsequent therapy modified according to findings at surgery. Patients who undergo laparotomy are randomized to receive one of the 3 following regimens: (1) limited field radiation therapy alone, (2) extended field radiation alone, or (3) limited field radiation therapy followed by chemotherapy using nitrogen mustard, vincristine, prednisone and procarbazine.

Twenty-seven patients have been entered into this study up to this point, for patients were randomized to receive no laparotomy and extended field radiation alone. All four obtained a complete remission and this persists to the present time (range 13-19 months). The remaining 23 patients all had laparotomies and were pathologically staged as I-IIA disease. Further randomization resulted in four patients having received involved field irradiation alone, 11 patients extended field irradiation alone and eight patients involved field irradiation plus MOPP chemotherapy. There have been six relapses up to this time, two among the patients receiving involved field irradiation alone at a relapse rate of 50% and four amongst the patients receiving extended field irradiation alone at a relapse rate of 36%. There have been no relapses among the involved field irradiation plus MOPP chemotherapy patients. An important component of the relapses observed amongst the patients receiving irradiation alone was that three of the relapses that have occurred or 50% of the patients receiving irradiation alone have been in patients with "E" stage disease of the lung. This high relapse rate following irradiation alone to such "E" stage patients confirms the findings previously indicated in an early study. It has, therefore, been decided that patients with "E" stage disease of the lung will no longer be included in this particular protocol. Although it is too early at this time to make any specific assessments of this particular study, it does appear at least that patients receiving irradiation plus chemotherapy are continuing to show improved results over those receiving irradiation alone.

Although staging laparotomy has been demonstrated to be of considerable benefit to many patients with Hodgkin's disease, there still exists controversy as to its value in patients with early localized Hodgkin's disease. This study should allow direct comparison of complication rates and overall survival in patients treated with irradiation alone who have undergone staging laparotomy as opposed to patients treated with irradiation alone who have not

undergone staging laparotomy in a prospectively randomized clinical trial. In addition, the possible benefits of adjuvant chemotherapy and/or extended field irradiation as opposed to involved irradiation should be evaluable, and may allow selection of the most effective and least toxic treatment regimen for patients with this potentially curable disorder.

Publications:

1. Levi, J.A. and Wiernik, P.H.: Unfavourable pronosis and therapeutic implications of limited extranodal Hodgkin's disease. Proceedings of XVIIIth Annual Meeting of the American Society of Hematology, Dallas, 1975, p. 60.

2. Levi, J.A., O'Connell, M.J.and Wiernik, P.H.: Patterns of relapse in stage I, II and IIIA Hodgkin's disease: Influence of initial therapy and implications for the future. Blood, (in press).

SMITHSONIAN CODING (IF AWARD) | PROJECT NUMBER (DO NOT use this space) | PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER: Z01 CM 06916-03 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (50 characters or less)
Pilot Protocol for Treatment of Patients with Stage III and IV Diffuse Histiocytic Lymphoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John A. Levi, M.D.	Clinical Investigator	CL NCI
	Peter H. Wiernik, M.D.	Head, Section of Med. Onc.	CL NCI
Other:	Stephen C. Schimpff, M.D.	Investigator	CL NCI
	Charles Diggs, M.D.	Clinical Associate	CL NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of this study have been to determine the value of high dose intensive chemotherapy for patients with diffuse histiocytic lymphomas utilizing measures to prevent infection which include laminar air flow isolation and prophylactic oral non-absorbable antibiotics. Patients with stage III and IV diffuse histiocytic lymphoma have been placed in reverse isolation in a laminar air flow unit, have commenced on prophylactic non-absorbable oral antibiotics, and given high dose chemotherapy with cyclophosphamide, adriamycin, vincristine and prednisone. Seven patients have been entered to date and in general, the tolerance to the high doses of chemotherapy have been good. Myelosuppression has generally occurred on days 10-14, after the initial administration of agents and, though often severe, has generally been brief. Results to date have been two pathologically confirmed complete responses and two patients who clinically achieved complete remission, but died as a result of sepsis during myelosuppression. There have been two failures on therapy, both patients having died towards the completion of their induction. One patient is currently too early for evaluation. The durations of remissions for the two pathologically confirmed complete responses have been 25+ months and 10 months, the second patient having eventually succumbed to cumulative cardiac toxicity from adriamycin therapy. Although the results of this study suggest that if patients are able to achieve complete response then long term duration of remission may be good, but the therapy is very aggressive and it still remains to be determined whether such approaches are justified.

Project Description:

To determine whether high dose intensive combination chemotherapy of patients with diffuse histiocytic lymphomas, utilizing measures to prevent infection such as laminar air flow isolation units and prophylactic oral nonabsnorbable antibiotics, will result in an improved remission rate and a prolonged survival.

Patients with biopsy proven malignant lymphoma of the diffuse histiocytic variety who have clinical stage III or stage IV disease are placed in reverse isolation in a laminar air flow unit, if available, and given prophylactic nonabsorbable oral antibiotics. These patients have been treated with doses of chemotherapeutic agents designed to produce significant myelosuppression in an attempt to provide better control of this devastating disease than is possible with conventional doses of the same drugs. The agents employed in this study are cytoxan, adriamycin, vincristine, and prednisone.

Seven patients have been entered onto the study to date. In general, tolerance to the high doses of chemotherapeutic agents employed was good and reverse isolation and the administration of the oral antibiotics has been well tolerated. Myelosuppression has generally occurred on days 10-14 after the initial administration of agents and although often severe, has generally been brief. Results of treatment to date have been two pathologically confirmed complete responses and two patients who clinically achieved complete remission but died as a result of sepsis during myelosuppression. There have been two failures on therapy, both patients having died towards the completion of their induction. One patient is currently too early for evaluation. The durations of remissions for the two pathologically confirmed complete responses have been 19+ months and 10 months; the second patient having eventually succumbed to cumulative cardiac toxicity from adriamycin therapy. Further patients are clearly needed to more adequately evaluate this study, but it does appear that there are definite problems in relationship to potential sepsis during the period of myelosuppression particularly if a patient is allowed out of a laminar air flow unit and also the concern of cumulative cardiac toxicity in patients receiving the combination of adriamycin and cyclophosphamide.

Disseminated diffuse histiocytic lymphoma is the most malignant variety of non-Hodgkin's lymphomas, with a median survival of less than one year from the time of diagnosis. Conventional chemotherapeutic regimens, although frequently producing some disease regression, are commonly ineffective in producing lasting disease control. Thus, if the infectious complications which are associated with severe bone marrow depression from chemotherapy can be avoided by the use of laminar air flow units and prophylactic oral nonabsorbable antibiotics, it may be possible to administer much higher doses of chemotherapy and thereby effect a more useful and long lasting remission of the disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 201 CM 36917-03 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

CAVP Versus CVP for Disseminated Non-Hodgkin's Lymphoma

NAMES, LABORATORY AND INSTITUTE OF PRINCIPAL INVESTIGATORS AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John A. Levi, M.D.	Clinical Associate	CL NCI
	Peter H. Wiernik, M.D.	Head, Section of Med. Onc.	CL NCI
Others:	Charles Digre, M.D.	Clinical Associate	CL NCI
	Charles Schiffer, M.D.	Senior Investigator	CL NCI
	Stephen Schimpff, M.D.	Senior Investigator	CL NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purposes of this study have been to determine the value of high dose intensive combination chemotherapy for patients with diffuse histiocytic lymphomas utilizing measures to prevent infection which include laminar air flow isolation and prophylactic oral non-absorbable antibiotics. Patients with stage III and IV diffuse histiocytic lymphoma have been placed in reverse isolation in a laminar air flow unit, have commenced on prophylactic non-absorbable oral antibiotics, and given high dose chemotherapy with cyclophosphamide, adriamycin, vincristine and prednisone. Seven patients have been entered to date and in general, the tolerance to the high doses of chemotherapy have been good. Myelosuppression has generally occurred on days 10-14, after the initial administration of agents and, though often severe, has generally been brief. Results to date have been two pathologically confirmed complete responses and two patients who clinically achieved complete remission, but died as a result of sepsis during myelosuppression. There have been two failures on therapy, both patients having died towards the completion of their induction. One patient is currently too early for evaluation. The durations of remissions for the two pathologically confirmed complete responses have been 25+ months and 10 months, the second patient having eventually succumbed to cumulative cardiac toxicity from adriamycin therapy. Although the results of this study suggest that if patients are able to achieve complete responsethen long term duration of remission may be good, but the therapy is very aggressive and it still remains to

Project Description: Z01 CM 06917-05 CL

1. To determine whether the addition of adriamycin to cyclophosphamide, vincristine, and prednisone will improve remission induction rates in patients with disseminated non-Hodgkin's lymphomas.

2. To evaluate the efficacy of maintenance chemotherapy in prolonging both remission duration and survival in those patients who achieve complete remission following induction therapy.

Patients who are found to have stage III or stage IV non-Hodgkin's lymphomas on the basis of clinical staging procedures are randomly assigned to receive therapy with either cyclophosphamide, 1000 mg/M^2 on day one IV, adriamycin 45 mg/M^2 on day one IV, vincristine 1.4 mg/M^2 on days 1 and 8 and prednisone 40 mg/M^2 on day 1 to 10 orally or cyclophosphamide 1500 mg/M^2 on day one IV, vincristine 1.4 mg/M^2 IV on days 1 and 8 and prednisone 40 mg/M^2 on days 1 to 10 orally without adriamycin for induction therapy. Patients who achieve complete remission then undergo consolidation therapy with CCNU, bleomycin and prednisone. A second randomization is then carried out with half the patients assigned to no further therapy and the remainder randomized to receive intermittent high dose cytoxan monthly intravenously until relapse occurs.

Thirty-two patients have been randomized to therapy to date, 18 patients to receive CAVP therapy and 14 patients CVP therapy. On the patients receiving CAVP, three are too early for evaluation, there have been 10 complete remissions (66%), two partial remissions and three failures. Mean duration of remission for complete responders has been 12+ months with a range of 1-27 months. There have been two relapses at 12 and 13 months. Of the 14 patients receiving CVP therapy, three are too early for evaluation, there have been six complete responses (55%), three partial responses and two failures. Their median duration of remission for the complete responders has been 14+ months with a range 1-17+ months and there has been one relapse at 2 months. Early results would, therefore, suggest that there is some advantage of the CAVP therapy although it is not clearly significant at this time. It is still too early to evaluate the efficacy of maintenance versus no maintenance therapy.

Conventional chemotherapy regimens have produced at best a 50% complete response rate in the non-Hodgkin's lymphomas, with the majority of patients ultimately relapsing and dying of progressive disease or complications of its therapy. Furthermore, an attempt to provide curative therapy, modeled after the approach which has been successful in acute lymphatic leukemia, employing intensive consolidation therapy is being studied. This study is designed to determine whether these results can be improved by adding the new antracycline antibiotic adriamycin to the induction therapy. Finally, it may be possible to determine the need for long term maintenance therapy in patients who achieve remission through the randomized scheme employed in this study.

Publications: None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06918-03 CL

PERIOD COVERED
July 1, 1975, through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Streptozotocin, Bleomycin, CCNU and Adriamycin Treatment of Advanced Hodgkin's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John A. Levi, M.D.	Clinical Associate	CL NCI
Other:	Peter H. Wiernik, M.D.	Head, Clinical Branch	CL NCI
	Charles Diggs, M.D.	Clinical Associate	CL NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study was to determine the clinical usefulness of a new combination chemotherapy for advanced Hodgkin's disease which is no longer responsive to conventional chemotherapy. Patients with advanced Hodkin's disease have received treatment with streptozotocin 0.5gm/M^2 daily for five days, CCNU 100mg/M^2 on day 1, bleomycin 15mg/M^2 IM on days 1 and 8, and adriamycin 45mg/M^2 on day 1 with cycles repeated each 28 days if blood counts permit. Seventeen patients have been currently entered onto this study and three are too early for evaluation and among the remaining 14 patients, there have been 4 complete remissions, 5 partial remission and 5 failures, a remission rate of greater than 50%. The duration of the unmaintained complete remissions have been 20+, 12, 9, and 6 months. This combination of agents appears to have definite efficacy in this previously treated group of patients and may, therefore, be of value in the subsequent treatment of all patients with advanced stage Hodgkin's disease whether or not they received prior chemotherapy.

PHS-6040

Project Description: Z01 CM 06918-03 CL

To determine the clinical usefulness of a new combination chemotherapy regimen for advanced Hodgkin's disease which is no longer responsive to conventional chemotherapy approaches.

Patients with advanced bipsy proven Hodgkin's disease who have failed conventional chemotherapy approaches are treated with a combination of streptozotocin 0.5mg/M^2 daily for 5 days, CCNU 100mg/M^2 on day 1, bleomycin 15mg/M^2 intramuscularly on days 1 and 8, and adriamycin 45mg/M^2 on day 1 with cycles repeated each 28 days if blood counts permit.

Seventeen patients have been currently entered onto this study. Three are too early for evaluation and among the remaining 14 patients there have been 4 complete remissions, 5 partial remissions and 5 failures, a remission rate of greater than 50%. The duration of the unmaintained complete remissions have been 20+, 12, 9 and 6 months. This combination of agents appears to have definite efficacy in this previously treated group of patients with Hodgkin's diase but it is too early as yet to draw firm conclusions.

Although currently available combination chemotherapy regimens, such as MOPP, have been shown to produce complete remissions in approximately 3/4 of patients treated there is still a sizable number of patients with previously untreated Hodgkin's disease who do not achieve remission utilizing these drugs. In addition, patients treated with radiation therapy for local disease occasionally relapse and a proportion of these patients prove refractory to standard chemotherapy approaches. Therefore, a need for an effective alternative regimen exists. Streptozotocin has been shown in preliminary stage II studies to have definite activity against refractory Hodgkin's disease and is an attractive agent for combination chemotherapy since it has a very mild bone marrow suppressive characteristic. Adriamycin, likewise, is a new agent which has been shown to have activity against Hodgkin's disease whose structure and mechanism of action is quite different than the agents commonly employed. Bleomycin has produced objective regressions in otherwise resistant Hodgkin's disease and it too has little bone marrow suppressive characteristics. CCNU has been shown to have significant activity in patients refractory to alkylating agents and vinca alkaloids. This, it is hoped that the combination of these four different chemotherapeutic agents with different mechanisms of action and different toxicities will be of benefit to patients with Hodgkin's disease who are not responsive to standard chemotherapy regimens.

PROJECT NUMBER (Do NOT use this space)

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CM-06919-02 CL

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (50 characters or less)

Initial Drug Therapy for Metastatic Renal Cell Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.M. Hahn	Clinical Associate	CL NCI
OTHER:	S.C. Schimpff	Senior Investigator	CL NCI
	P.H. Wiernik	Chief, Section of Medical Oncology	CL NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Branch

SECTION

Medicine

INSTITUTE AND LOCATION

NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.50	0

SUMMARY OF WORK (200 words or less - underline keywords)

Because renal cell carcinoma is notoriously resistant to chemotherapy and radiation, occasionally cured by surgery, a prospective randomized controlled chemotherapy trial was established to compare efficacy of pO. CCNU, IV vinblastine, or IV ThioTepa. Those patients failing to respond to first line form of therapy will then receive IM bleomycin. So far, 18 patients with metastatic renal cell carcinoma have been randomized between the three first line forms of therapy. The three groups are equivalent in age, prior therapy, time from diagnosis to therapy and incidences of CNS metastases. Eight patients were treated with CCNU and there were no partial responses, two stabilizations and four progressions. Four patients received vinblastine. Vinblastine and its therapy resulted in one partial remission, two stabilizations and one progression. ThioTepa treatment was given to 5 patients in one partial remission, 2 stabilizations and 3 progressions. Mild to moderate suppression was a major side effect for each drug regimen, emesis occurred only in the CCNU group. Survival time was equivalent for all 3 groups and did not differ from historical controls. Brain metastases were associated with early deaths in 3 out of 4 patients. Of the 3 patients who received bleomycin, there were no partial responses one stabilization and 2 progressions. The protocol will be continued, as is presently written, with 1 exception, the CCNU arm has been closed.

PHS-6040
(12-70)

(888)

Project Description:

Objectives:

Renal cell carcinoma is a solid tumor of notorious resistance to chemotherapy and radiation. Surgery may be curative in some cases, the overwhelming percentage of cases proceed to metastatic disease and death within two years of diagnosis. Most previous chemotherapy studies have been inadequate. Several previous well controlled studies indicated that adriamycin had no activity and provera had only minimal activity. Drugs that have shown some efficacy in preliminary trials included CCNU with a 10 to 15% response rate, vinblastine with approximately the same response rate and bleomycin which had less of a response rate. In addition, a drug derivative of thiotepa which had been tried in the Soviet Union, was very promising with approximately 50% response rate. Therefore, a study was set up to randomize between vinblastine, CCNU and thiotepa for the first line of therapy and if the patient relapsed from this therapy then a second round of therapy would be bleomycin unless there was sufficient reason not to give the bleomycin because of chronic obstructive pulmonary disease.

Major Findings:

So far, 18 patients with metastatic hypernephroma have been randomized between the 3 first line forms of therapy. Three groups are equivalent in age, prior therapy, and time from diagnosis to therapy and incidences of CNS metastases. Eight patients were treated with CCNU therapy. This therapy resulted in no partial responses, 2 stabilizations and 4 progressions. Vinblastine therapy was received by 4 patients and resulted in one partial remission, 2 stabilizations and 1 progression. Thiotepa treatment was given to 5 patients as primary therapy and resulted in 1 partial remission, 2 stabilizations and 3 progressions. Myelosuppression was a major side effect in each drug with no complication other than drug therapy delay, resulted from this. Emesis occurred in the CCNU group in all 8 patients. Survival time was equivalent in the three regimens and did not differ from historical controls. Brain metastases were associated with early deaths in 3 out of 4 and a lack of response to therapy. Those patients surviving initial therapy were treated with bleomycin. At this point, only 3 people have been tried in the bleomycin regimen resulting in 0 partial responses, 1 stabilization and 2 progressions. At present, the CCNU arm has been dropped out and patients are now being randomized between vinblastine and thiotepa and all patients except for those with chronic obstructive pulmonary disease, if they fail the first line regimen, are being placed on the bleomycin regimen.

Publications:

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06920-03 CL

PERIOD COVERED
July 1, 1976 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

New Drug Therapy of Previously Treated Adult Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John A. Levi, M.D.	Clinical Associate	CL NCI
	Peter H. Wiernik, M.D.	Chief, Section of Med. Onc.	CL NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The results of this study indicate that Azacytidine is a useful drug for the treatment of previously treated and relapsed patients with acute nonlymphocytic leukemia. In such patients approximately 30% complete remission rates can be expected with this drug. Guanazole was shown to have no significant activity in such patients.

PHS-6040
(12-75)

Project Description: Z01 CM 6920 03 CL

To determine the relative efficacy of two new experimental chemotherapeutic agents, 5-azacytidine and guanazole, in the treatment in advanced adult acute non-lymphocytic leukemia which is no longer responsive to conventional chemotherapeutic agents.

Thirty patients were entered into this study. Eighteen received 5-azacytidine and 6 achieved remission, 5 of which were complete (28%). The median duration of complete remission was 100 days. Twelve patients received therapy with guanazole and only one achieved partial remission. Principal toxicities encountered with 5-azacytidine therapy included gastrointestinal intolerance, feber and neuromuscular toxicity. Fever was the principal toxicity observed after guanazole therapy, one patient developed erythema nodosum and another recurrent pulmonary infiltrate following guanazole therapy. 5-asacytidine therefore has significant activity as an induction agent in ANLL, but guanazole appears to have no benefit for these patients.

Publications:

Levi, J.A., Wiernik, P.H.: A Comparative Clinical Trial of 5 Azacytidine and Guanazole in Previously Treated Adults with Acute Nonlymphocytic Leukemia: Cancer (in press) 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06921-03 CL

PERIOD COVERED
July 1, 1975, to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Adriamycin Therapy for Advanced Mycosis Fungoides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	:	J. A. Levi	Sr. Clin. Assoc.	CL	NCI
		P. H. Wiernik	Head, Clin. Br.	CL	NCI
Other	:	C. Diggs	Sr. Clin. Assoc.	CL	NCI
		R. A. Slawson			

COOPERATING UNITS (if any)
Section of Radiotherapy, University of Maryland Hospital, Baltimore, Md.

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study has been to determine the efficacy of adriamycin therapy for the teatment of advanced mycosis fungoides. Adriamycin has been administered to patients with a biopsy proven diagnosis of mycosis fungoides who were refractory to any prior therapy. Disease status was advanced in all patients with extensive skin involvement and lymph adenopathy and frequent visceral disease. Adriamycin was administered in a dose of 60mg/M^2 IV every three weeks and a minimum of three courses given beyond complete response. Maintenance therapy consisted of methotrexate 15mg/M^2 IM twice weekly and cytoxan 750mg/M^2 IV every three weeks. Thirteen patients have been entered into this study and there have been three complete responses (23%), five partial responses (39%) and three improvements (23%) and two failures. The median duration of response for the complete responders has been 32+ weeks, partial responders 20+ weeks and improvement 18 weeks. Toxicity has been mild and it appears that adriamycin is an active agent for the treatment of advanced mycosis fungoides.

Project Description: Z01 CM 06921-03

To determine the efficacy of adriamycin therapy for the treatment of advanced mycosis fungoides.

Adriamycin was given to patients with a biopsy proven diagnosis of mycosis fungoides who were admitted to the BCRC. All patients had received previous therapy and were now refractory. Disease status was advanced, extensive skin disease, lymphadenopathy was present in all cases. Adriamycin was administered in a dose of 60 mg/M^2 IV every 3 weeks and doses modified if any liver dysfunction or significant myelosuppression occurred. A minimum of 3 courses were given and if complete response ensued, a further 2 courses beyond complete response given. Maintenance therapy consists of methotrexate 15 mg/M^2 IM twice weekly and cytoxan 750 mg/M^2 IV every 3 weeks.

Thirteen patients have been entered into this study and all are evaluable. There have been 3 complete responses (23%), 5 partial responses (39%), three improvements (23%) and two failures. The median duration of response for the complete response responders has been 32+ weeks (10+ to 32+ weeks), partial responders 20+ weeks (8 to 108 weeks) and improvements 18 weeks (8 to 22 weeks). Toxicity has been mild and only a moderate degree of myelosuppression encountered. It, therefore, appears that adriamycin is an effective agent for the treatment of advanced mycosis fungoides.

Mycosis fungoides is an unusual form of lymphoma which often has a slow course early in its stages of development. However, once the disease is advanced, there is often rapid progression and no form of chemotherapy used to this time has improved survival. Adriamycin appears to be an effective agent and if intensive therapy with this drug can produce a number of responses and prolong duration of response and survival time, then further studies with this agent alone and in combination with others may improve the outlood for patients wit this distressing disease.

Publications:

1. Levi, J. and Wiernik, P.: Management of mycosis fungoides - Current status and future prospects. Medicine 54: 73, 1975.

2. Levi, J.A. and Wiernik, P.H.: Adriamycin therapy for advance mycosis fungoides. Proc. of the A.A.C.R., 1976, (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06922-04 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Epidemiology Investigations of Patients with Leukemia and Lymphoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Senior Investigator	CL NCI
OTHER:	D.A. Brager	Research Associate	CL NCI
	P.H. Wiernik	Chief, Section of Medical Oncology	CL NCI

COOPERATING UNITS (if any)

G. Comstock Johns Hopkin's University School of Public Health and Hygiene

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.25	.75

SUMMARY OF WORK (200 words or less - underline keywords)

Recent published observations have suggested that some patients with Hodgkin's disease may have been close personal contacts with others who subsequently developed the disease. The BCRC investigation was instituted to assess the possible relevance of interpersonal contact in the epidemiology of both leukemia and lymphoma. Following initial evaluations which suggested a high frequency of personal interactions among patients prior to diagnosis, two separate areas were then chosen for case-control evaluation of social linkages. In one of the two areas, the cases had significantly more close personal associations than expected thus supporting, in part, the previous observations of social linkages between patients.

PHS-6040
(12-75)

Project Description: Z01 CM 06922 04 CL

Prior Social Interaction Among Patients with Leukemia and Lymphoma- Recent published observations have suggested that some patients with Hodgkin's disease may have been close personal contacts with others who subsequently developed the disease. The BCRC investigation was instituted to assess the possible relevance of interpersonal contact in the epidemiology of both leukemia and lymphoma. Each case report of leukemia and lymphoma for a 10-year period from three areas of West Virginia was obtained and the patient or a surviving friend or relative was interviewed. In each area, over 60% of all cases could be interlinked on the basis of prior close personal interactions. Similar findings were obtained from two areas in Maryland and one in Pennsylvania.

Two separate areas were then chosen for case-control evaluation of social linkages. From each area, every patient with leukemia or lymphoma was ascertained through the tumor registries. Controls were randomly selected, matched for age, sex, and residence as of date of diagnosis. Social linkages between cases, controls and cases plus controls were ascertained. In one of the two areas, the cases had significantly more close personal associations than expected thus supporting, in part, the previous observations of social linkages between patients. This case-control approach will be extended to other areas.

Publications:

Schimpff, S.C., Brager, D.M., Schimpff, C.R., Comstock, G.W., and Wiernik, P.H.: Leukemia and lymphoma patients linked by prior social contact. Evaluation utilizing a case-control approach. Ann. Int. Med. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06928 02 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
High Dose Methotrexate (HDMTX) for the Therapy of Lymphoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. A. Schiffer	Sr. Investigator	CL NCI
	J. A. Levi	Clin. Assoc.	CL NCI
	P. H. Wiernik	Head, Clin. Br.	CL NCI
Other:	S.D. Reich		CL NCI
	N. Bachur	Head, Biochem.Sect.	CL NCI

COOPERATING UNITS (if any)
Biochemistry Section, BCRC

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

High Dose Methotrexate followed by citrovorum factor "rescue" has been administered to patients with refractory lymphoma and advanced sarcoma. In order to decrease toxicity, bicarbonate is administered orally and urinary pH is monitored. Radioimmunoassay for methotrexate has been used and will hopefully be of assistance in regulating dosage in the future. Todate, .2 with advanced lymphoma have been treated and neither have responded to therapy. There have been no objective responses in patients with metastatic soft tissues sarcomas.

PHS-6040
(12-75)

1. To determine the efficacy of high dose methotrexate in patients with advanced, refractory lymphoma and solid tumors.

2. To determine the feasibility of administering high dose methotrexate to patients who have undergone extensive prior therapy.

Accrual of patients with refractory lymphoma has been slow. In the past year, three additional patients with soft tissue sarcoma have been treated, none of whom have had either complete or partial remission of disease. One patient with metastatic osteogenic sarcoma continued to remain stable with metastatic pulmonary disease.

The protocol has been altered somewhat and now all patients receive their citrovorum factor intravenously for two days in order to insure adequate fluid intake and high urine output. In addition, the urine pH is closely monitored and bicarbonate is administered to assure adequate urinary alkalinization so as to increase methotrexate excretion and decrease systemic toxicity.

A radioimmunoassay for methotrexate has been used which closely parallels the results achievable by bio-assay. This should enable closer regulation of methotrexate and citrovorum doses in the future.

In view of the absence of response in metastatic sarcoma, patients with this tumor will no longer be treated with high dose methotrexate. The protocol will remain active for patients with advanced lymphoma.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06929-02 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Combination Therapy in Previously Treated Adults with Acute Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John A. Levi, M.D.	Clinical Associate	CL NCI
	Peter H. Wiernik, M.D.	Chief, Section of Med. Onc.	CL NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

In this study, methyl GAG was added to 5-Azacytidine for induction therapy of patients with acute nonlymphocytic leukemia who have become refractory to more standard agents. It was determined that the addition of methyl GAG added nothing to the activity of 5-Azacytidine

PHS-6040
(12-75)

Project Description: Z01 CM 06929-02 CL

To determine whether the combination of 5-azacytidine and methyl GAG could improve on the results achieved with 5-azacytidine alone and whether administration of methyl GAG as a continuous IV infusion will reduce the incidence of serious toxicity previously reported with this drug.

Eight adults with acute nonlymphocytic leukemia who have received extensive prior therapy were included in this study. Only one patient achieved a partial remission on this regimen and one other achieved definite improvement in bone marrow status without approaching a remission. Survival from the onset of therapy was brief for the remaining six patients (2 to 8 weeks). All patients treated with this regimen suffered definite and often severe nonhematologic toxicity. The addition of methyl GAG resulted in an increase in both the number and degree of severity of toxic effects previously described with 5-azacytidine alone. Mucosal toxicity, in particular, was a serious problem and could be directly related to subsequent infection and death of four patients. Because of the poor responses and severe toxicity encountered with this regimen, it is felt that this combination is not suitable for further study. It also seems that no advantage could be obtained from the continuous IV infusion of methyl GAG and this drug in general remains too toxic to use in induction of acute leukemia.

Study closed to patient entry and other combination studies with 5-azacytidine have begun.

Publications:

1. Levi, J., Wiernik, P.H.: Combination Therapy with 5-Azacytidine and Methyl Glyoxal Bis Guanylyhydrazone in Previously Treated Adults with Nonlymphocytic Leukemia. Can Chem Rep 59: 1043-1045, 1975.

2. Levi, J.A., Wiernik, P.H.: A Comparative Clinical Trial of 5-Azacytidine and Guanazole in Previously Treated Adults with Acute Nonlymphocytic Leukemia: Cancer (in press), 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06939-01 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Methotrexate, Oncovin, L'Asparaginase, and Dexamethasone Therapy for Adults with ALL

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert J. Esterhay, Jr., M.D.	Senior Investigator	CL NCI
Others:	A. Collier Smyth, M.D.	Clinical Associate	CL NCI
	Peter H. Wiernik, M.D.	Head, Clinical	CL NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	1

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to improve the remission induction rate of acute lymphocytic leukemia in adult patients utilizing vincristine and dexamethasone with intermittent moderate doses of methotrexate followed by L'asparaginase. The second objective is to determine whether intermittent high dose methotrexate with leucovorum rescue and with vincristine and dexamethasone will prolong remission duration. The third objective is to determine the efficacy of moderate and high dose methotrexate in preventing meningeal leukemia and to correlate the clinical effectiveness with the cerebral spinal fluid methotrexate levels. To date, no previously untreated patients have been entered onto this study. Five previously treated patients have been entered on the study. Two patients achieved a complete remission after one course, one patient obtained a partial remission after three courses, and two patients are too early to evaluate (they both have started their first course of therapy).

PHS-6040
(12-75)

The purpose of this project is to improve the remission induction rate of Acute Lymphocytic Leukemia in adult patients utilizing vincristine and dexamethasone with intermittent moderate doses of methotrexate followed by L'asparaginase. The second objective is to determine whether intermittent high dose methotrexate with leucovorum rescue and with vincristine and dexamethasone will prolong remission druation in a significant percentage of adult patients with Acute Lymphocytic Leukemia. The third objective is to determine the efficacy of moderate and high dose methotrexate in preventing meningeal leukemia and to correlate clinical effectiveness with the cerebral spinal fluid methotrexate levels.

Patients over the age of 15 years and over with a diagnosis of Acute Lymphocytic Leukemia will be eligible. Induction therapy will consist of methotrexate 100 mg/M^2 IV push on day one only, dexamethasone 6 mg/M^2 orally daily, vincristine 2 mg (regardless of body surface area) IV push on day two only and L'asparaginase 500 IU/kg IV infusion over a 30 minute period on day two only. The induction therapy is to be continuous with the second course beginning on day eleven, the third course on day 21, etc. Patients achieving a complete remission precede to consolidation therapy which consists of continuing with only methotrexate and L'asparaginase. The consolidation therapy consists of six cyclic courses which begins the day after the completion of induction therapy. Patients remaining in complete remission after six courses of consolidation therapy will proceed to cytoreduction therapy. Cytoreduction therapy consists of vincristine, dexamethasone, high dose methotrexate (100 mg/kg) followed by citrovorum factor (5% of the high dose methotrexate dose (mg/mg) over three days). Cytoreduction therapy is given monthly for a total of twelve courses. Maintenance therapy begins after the completion of cytoreduction and is continued indefinitely until bone marrow relapse with each course given at four week intervals. Maintenance therapy consists of vincristine on day one, dexamethasone orally on days one through five, methotrexate (15 mg/M^2) orally weekly, and 6-mercaptopurine orally daily.

No previously untreated patients have been entered onto this study as of yet. Five previously treated patients have been entered on the study. Two patients achieved a complete remission after one course, one patient has obtained a partial remission after three courses and two patients are too early to evaluate (starting first course of therapy).

Publications:

1. Smyth, A.C. and Wiernik, P.H.: Combination chemotherapy of Adult Acute Lymphocytic Leukemia. Clinical Pharmacology and Therapeutics 19: 240-245, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06940-01 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
Cost Analysis of Leukemia Treatment: A Problem Oriented Approach

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert J. Esterhay, Jr., M.D.	Senior Investigator	CL NCI
Others:	Peter H. Wiernik, M.D.	Head, Clinical Branch	CL NCI
	Clarence L. Fortner	Pharmacist	CL NCI
	Victor G. Vogel, M.D.		

COOPERATING UNITS (if any)
Clinical Research Pharmacy Service

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

SUMMARY OF WORK (200 words or less - underline keywords)

A Problem Oriented List of Charges (POLC) can be used to obtain information about the cost of treatment of a single medical problem in patients with several co-existing medical problems. To provide a POLC, each charge is associated with one of the patient's problems. POLC's were compiled from the Problem Oriented Medical Records (POMR's) of patients with Acute NonLymphocytic Leukemia (ANLL). From the POLC's it was apparent that the treatment of granulocytopenia and thrombocytopenia and their sequelae were the most costly problems associated with ANLL. The projected cost of treatment for all problems for 11.5 months (the median survival of patients with ANLL treated with a single chemotherapeutic agent at this institution) was $40,022. By extending the POMR into the area of medical reimbursement with the POLC, charge incurring activities can be coupled to the results of patient care. Only by directly coupling in the original medical record, the reasons for, the charges for, and the results of medical care, can cost data be appropriately interpreted.

PHS-6040
(12-75)

Project Description: Z01 CM 06940-01 CL

The objective of the project was to couple charges in the medical record to the problem under which they incurred so that for any particular problem the total charge could be assessed. Specifically, the objectives were to provide a cost analysis of the treatment of acute non-lymphocytic leukemia and thereby a model for general medical application.

The Problem Oriented Medical Record (POMR) was used to compile a Problem Oriented List of Charges (POLC) for adult patients with acute non-lymphocytic leukemia (ANLL) POLC's were prepared byassociating each charge item with one of the patient's problems.

From the POLC's, it was apparent that the treatment of granulocytopenia and thrombocytopenia and their sequelae were the most costly problems associated with ANLL. The projected cost of treatment for all problems for 11.5 months (the median survival of patients with ANLL treated with a single chemotherapeutic agent at BCRC) was $42022.

By extending the POMR into the area of medical reimbursement with a POLC, charge incurring activities can be coupled to the results of patient care. Only by directly coupling, in the original medical record, the reasons for, the charges for, and the results of medical care, can cost data be appropriately interpreted. Through the use of problem-specific charges over a defined population it will become possible to determine the long term cost of treating specific problems. This data could be used for cost/benefit analysis of different cancer treatment protocols.

Proposed Course:

Study concluded.

Publications:

1. Esterhay, R.J., Jr., Vogel, V.G., Fortner, C.L., Shapiro, H.M., Wiernik, P.H.: Cost Analysis of Leukemia Treatment - A Problem Oriented Approach. Cancer 37: 646-652, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06941-01 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)
High Dose 6-Mercaptopurine Therapy for Advanced, Refractory Neoplastic Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert J. Esterhay, M.D.	Senior Investigator	CL NCI
Others :	Joseph Aisner, M.D.	Senior Investigator	CL NCI
	John A. Levi, M.D.	Clinical Associate	CL NCI
	Peter h. Wiernik, M.D.	Head, Clinical Branch	CL NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Branch

SECTION
Medicine

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

SUMMARY OF WORK (200 words or less - underline keywords)

There are few protocols that encorporate 6-mercaptopurine at a dose level of 500-100mg/M^2 other than those that have been used to treat acute lymphocytic leukemia in children and acute nonlymphocytic leukemia in adults. In such studies count suppression, nausea and vomiting and moderately severe mucositis and stomatitis have occurred. 6-mercaptopurine has not been tested clinically as a single agent in high doses with respect to its antitumor effect. The objective of this study is to examine the antitumor effect of high dose 6-mercaptopurine in a broad spectrum of disseminated refractory neoplastic diseases. This is a non-randomized study and all patients received high dose 6-mercaptopurine 1000mg/M^2 per day given by rapid IV infusion o n the first five days of each 21 day course. As of February, 1976, 20 patients have been entered onto this study. There have been no complete responses and there have been no partial responses. Four patients, all who had breast cancer, had objective responses (a response less than a partial response), two patients, both with lung cancer, had stabilization or no change in their disease and they had four or more courses of therapy. None of the 20 patients entered on this study are alive at this time. Hematologic toxicity was uniformly severe with count suppression occurring in all patients; in addition, nausea and vomiting occurred in all patients as well as moderate to severe mucositis and stomatitis.

PHS-6040
(12-75)

There are few protocols that incorporate 6-mercaptopurine at a dose level of 500-1000 mg/M^2 other than those that have been used to treat acute leukemia. High dose 6-mercaptopurine has been used in the POMP combination therapy protocol utilizing high dose 6-MP with prednisone, vincristine and methotrexate for treating acute lymphocytic leukemia in children and acute non-lymphocytic leukemia in adults. In both studies count suppression, nausea and vomiting, and moderately severe mucositis and stomatitis. 6-Mercaptopurine has not been tested clinically as a single agent in high doses with a respect to its anti-tumor affect.

The objective of this study is to examine the anti-tumor affect of high dose 6-mercaptopurine in a broad spectrum of disseminated refractory neoplastic diseases. Any patient with microscopically confirmed malignant disease not otherwise emendable to conventional therapy is eligible for the study. The patient must have measurable disease parameters clinically and/or radiographically and should have a life expectancy of two months or more. The patient should have a white count greater than 4,000 and a platelet count greater than 100,000 except where low counts are disease related. No chemotherapy or radiotherapy should have been given for two weeks prior to the start of high dose 6-mercaptopurine. Since high dose 6-mercaptopurine is excreted to the liver and kidney, the functions of these organs should be adequate (bilirubin less than 1.2 mg/dl, serum creatinine less than 1.5 mg/dl prior to entering a patient into this study.

This is a non-randomized study and all patients receive high dose 6-mercaptopurine, 1000 mg/M^2/day given by rapid IV infusion on the first 5 days of each 21 day course. Subsequent doses are decreased according to toxicity. All patients receive a minimum of two courses of theapy and three courses if progression of disease does not occur during therapy. For those patients responding to therapy at least three additional courses of treatment are given after complete remission is achieved or a minimum of six courses. Patients whose disease progresses after two courses, respond partially but stabilize at the residual disease or show no change after three courses of therapy are taken off the study. Patients who progress with toxicity are off study and patients who achieve a complete or partial continue therapy to relapse at which time they are off study.

As of February, 1976, 20 patients have been entered onto this protocol. There have been no complete responses and there have been no partial responses. Four patients, all who had breast cancer, had objective responses (in response less than a partial remission), two patients, both with lung cancer, had stabilization or no change in their disease and they had four or more courses of therapy. None of the patients are alive at this time. However, at the writing of this project description, two additional patients have been added onto this study, and it is too early to evaluate their responses. Hematologic toxicity was uniformly severe with count suppression occurring in all patients, nausea and vomiting occurred in all patients as well as moderately mucositis and stomatitis.

Proposed Course:

Conclude this investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06942-01 CL

PERIOD COVERED

July 1, 1975, through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Development of the Acoustic Microscope as a Useful Tool in Hematology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Medical Oncology Section	CL NCI
Other:	C. F. Quate, Ph.D.	Professor of Applied Physics and Electrical Engineering	Stanford, Univ. Stanford, Ca.

COOPERATING UNITS (if any)

Stanford University, Stanford, California

LAB/BRANCH

Clinical Branch

SECTION

Medicine

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The acoustic microscope, which utilizes microwave sound rather than light or electron beams to reproduce an image, is investigated in this study from its possibility as a useful tool in hematologic research. Early results indicate that appropriate resolution is possible.

PHS-6040

Project Description:

The acoustic microscope, developed at Stanford University, utilizes microwave sound rather than light or electrons to reproduce the image of a microscopic object. Objects to be examined by the instrument need no fixing or staining. We are endeavoring to adapt this instrument for the examination of peripheral blood and bone marrow by addressing ourselves to problems of resolution. Early results are encouraging that this instrument may ultimately be useful for rapid examination of hematologic specimens.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06942-01 CL

PERIOD COVERED

July 1, 1975, through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Development of the Acoustic Microscope as a Useful Tool in Hematology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Medical Oncology Section	CL NCI
Other:	C. F. Quate, Ph.D.	Professor of Applied Physics and Electrical Engineering	Stanford, Univ. Stanford, Ca.

COOPERATING UNITS (if any)

Stanford University, Stanford, California

LAB/BRANCH

Clinical Branch

SECTION

Medicine

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY OF WORK (200 words or less - underline keywords)

The acoustic microscope, which utilizes microwave sound rather than light or electron beams to reproduce an image, is investigated in this study from its possibility as a useful tool in hematologic research. Early results indicate that appropriate resolution is possible.

Patients with either primary or secondary neoplasms effecting the central nervous system are referred to the Baltimore Cancer Research Center Section of Neurological Surgery for combined modality therapy. Since our move to the University of Maryland Hospital, this service has operated an active consultation service to the entire hospital for patients with tumors effecting the nervous system and we have had an opportunity to see and treat a wide variety of both primary and secondary neoplasms in such a manner. Since the clinical and pharmacological evaluation of chemotherapeutic agents is one of the principal aims of this service, we have sought this year to improve the quality of evaluation of our patients with intracranial neoplasms by utilizing the E.M.I. Scanner at the University of Maryland Hospital. This new device has added precision to the assessment of tumor dimensions and has thus contributed greatly to the assessment of experimental drug therapy.

Previous studies at this center have indicated the efficacy of both intravenously and orally administered nitrosourea compounds and epipodophyllotoxin. A Phase II study utilizing these drugs in relation to radiotherapy has been completed. A Phase II study of dianhydrogalactitol in the treatment of primary malignant gliomas is nearing completion. A pilot study utilizing piperazine dione in the treatment of refractory malignant brain tumors has just been instituted. In addition, the neurosurgical service cooperates with, and participates with, in the brain tumor study group.

The close association that this service enjoys with the Medical Oncology Service at the BCRC has been advantageous to both. We have been interested for the past few years in new approaches to the treatment of meningeal neoplasia. A Phase II study using ThioTepa in the treatment of meningeal carcinomatosis and meningeal leukemia is nearing completion. In addition, our experience with the use of cytosine arabinoside in the treatment of both lymphocytic and non-lymphocytic leukemic meningitis and meningeal carcinomatosis is currently being evaluated. As the treatment of the leukemias becomes more successful and as patients survive a longer time, we are seeing more and more patients develop these late complications of their malignancy and this has provided increasing numbers of patients for evaluation.

In addition to the combined modality approach of radiotherapy and chemotherapy, the Neurosurgical Service at the BCRC continues to re-operate on selected patients with malignant brain tumors. This multimodality approach of therapy in the treatment of intracranial neoplasms continues to be our approach to this very urgent problem.

Publications:

1. Walker, M.D.: Diagnosis and Treatment of Malignant Brain Tumor. Ca - A Cancer Journal for Clinicians, 25, No. 3, 114-120, May/June 1975.

2. Fortner, C.L., Grove, W.R., Bowie, D. and Walker, M.D.: Fat Emulsion Vehicle for Intravenous Administration of an Aqueous Insoluble Drug. J. Hosp. Pharmacy, 32: No. 6, 582-584, June 1975.

3. Walker, M.D.: Recent Advances for Brain Tumors, Cancer Chemotherapy - Fundamental Concepts and Recent Advances, Chicago, Yearbook Medical Publishers, 251-262, 1975.

4. Walker, M.D. and Weiss, H.D.: Chemotherapy in the Treatment of Malignant Brain Tumors, Advances in Neurology, Vol 13: 149-153. Edited by W.J. Friedlander, New York, Raven Press, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06282-09 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Brain Tumor Study Group

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael D. Walker, M.D.	Associate Director, BCRC	CL,NCI
	T. A. Strike	Sp. Assist. Prog. Dev.	CL,NCI

COOPERATING UNITS (if any)

Members of the Brain Tumor Study Group

LAB/BRANCH
Clinical Branch

SECTION
Neurosurgery

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Mayralnd 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

University affiliated investigators, collectively known as the Brain Tumor Study Group, are cooperating in a continuous effort -- conducting Phase III clinical trials to evaluate chemotherapy, radiation therapy and combinations thereof (multi-modality therapy) following definitive surgery in the treatment of brain tumors (malignant gliomas). New chemotherapeutic agents are also being tested in Phase II clinical trials in order to make the selection of chemotherapeutic agents for future Phase III trials more meaningful.

In addition to testing the efficacy of various treatments in malignant brain tumor, radiation effects and drug toxicity are being assessed. Data is also being accumulated that will provide a basic understanding of the biology, natural course and peculiarities of the disease entity under investigation.

Work completed has shown that radiation therapy alone and in combination with the nitrosoureas significantly extends the median survival of brain tumor patients.

Project Description: Z01 CM 06282-09 CL

The Brain Tumor Study Group (BTSG) was established in order to provide a rapid, scientific and controlled method of evaluating chemotherapy in the treatment of malignant gliomas utilizing Phase III type clinical trials. The goals of the BTSG are (1) to increase the number of patients that respond to treatment, (2) prolong the period of remission, and (3) increase the life expectancy of these patients. Since inception, the program has been broadened to include evaluation of radiation therapy as a modality alone and in combination with chemotherapy. Additionally, the BTSG is also conducting Phase II studies in order to make a more meaningful selection of chemotherapeutic agents for future Phase III studies.

Members of the Brain Tumor Study Group have established a cooperative, ongoing and continuous effort providing patients for multi-therapeutic modality studies which utilize a prospective, controlled, randomized study design. Patients diagnosed for the first time as having malignant gliomas are randomized to the Phase III study shortly after definitive surgery. Patients with recurrent gliomas or other types of brain tumors are randomized to the Phase II study.

A paper summarizing the results of the Phase III study evaluating BCNU and radiation therapy alone and in combination is being reviewed prior to submission for publication. Of the 303 patients randomized into the four treatment arms, 225 were valid study patients. Results of this study indicated that radiation therapy alone and radiation therapy in combination with BCNU significantly increased the survival time of patients with malignant gliomas. However, the difference in survival time between these treatments was not statistically significant.

ThePhase III clinical trial designed to evaluate MeCCNU alone and in combination with radiation therapy was closed to accrual on December 31, 1975. Of the 467 patients randomized to this study, approximately three-quarters of them are expected to be valid study patients. Since approximatley 40% of the study patients are still living, final data analysis for this study is probably a year away. Preliminary analysis of the current data base indicates an estimated median survival of 51 weeks for patients receiving BCNU plus radiation therapy as compared with those receiving radiation therapy alone (36 weeks), methyl CCNU alone (31 weeks) and methyl CCNU plus radiation (31 weeks).

On January 1, 1976, the BTSG initiated a new Phase III protocol. This study was designed to evaluate both the oncological effect and effect on survival of high doses of corticosteroids in brain tumor patients. The best treatment arm from the previous Phase III study constitutes the control, while steroid alone, steroid plus BCNU and procarbazine alone will be administered to malignant glioma patients following definitive surgery and concomitant radiation theerapy. Accrual to date has been excellent.

The Phase II study which was testing adriamycin, dibromodulcitol, procarbazine and streptozotocin in brain tumor patients has almost reached the point where it can be closed to accrual. Preliminary results indicate that the median survival time of procarbazine treated patients was marginally better than those of the other drugs being tested. Procarbazine was, therefore, moved into the current Phase III trial. A new Phase II protocol is being drafted and will be initiated soon.

During the past year the BTSG expanded its membership. As a result of the RFP for group expansion, four University medical centers were awarded contracts and became members. During the year the eight existing BTSG contracts were openly recompeted -- with the loss of one former member and the addition of two new members. The BTSG now appears to have the potential of completing a Phase III study every two years.

The Fall meeting of the BTSG consisted of a business session in which the details of the proposed Phase III study were finalized and a scientific session in which the theme was "The Detection of Recurrence in the Brain Tumor Patient Using Computer Assisted Tomography Scanning." The Spring meeting was devoted entirely to business matters concerned with a Phase II protocol, discussion of drug toxicity and other topics of immediate interest to the members of the BTSG.

The BTSG studies served as the prototype for the several, recently inaugurated, cooperative efforts utilizing prospective, controlled, randomized studies for the treatment of malignant brain tumor. The BTSG studies are generating specific, detailed and timely information concerning the efficacy of the various therapeutic modalities utilized, as well as insight into the biological characteristics of the disease entity being investigated. This project is also in keeping with both the long range goal and short term objective of the Division of Cancer Treatment program.

Publications:

1. Walker, M.D., et al*: The evaluation of mithramycin (NSC-24599) in the treatment of anaplastic gliomas. J. of Neurosurg. 44: , 1976.

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* All principal investigators of the BTSG.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06904-04 LPH

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Anti-Tumor Activity of Nitrosoureas at the Subcellular Level

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Hilton, Ph.D.	Visiting Associate	LPH, NCI
Others:	Michael D. Walker, M.D.	Associate Director, BCRC	CL, NCI
	Philip H. Gutin, M.D.	Clinical Associate	CL, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Neurosurgery

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

SUMMARY OF WORK (200 words or less - separate from this form)

This project is studying the effects of nitrosourea anti-tumor agents at the subcellular level. The current investigations are related to: (1) cell respiration; (2) damage to cellular DNA; and (3) repair of DNA damage.

These studies have concentrated on the mechanism of action of CCNU since there is considerable information available on the metabolism (hydroxylation) of CCNU both in vivo and in vitro (Z01 CM 06911-03 BCRC; Z01 CM 06910-03 BCRC).

We have previously described the appearance of single-strand breaks in L1210 cells in vivo and in tissue culture following exposure to low concentrations of CCNU (Z01 CM 06904-03 BCRC). This report describes recent work in pursuit of the mode of action of the nitrosourea anti-tumor agents.

1. Effect of nitrosoureas on cellular respiration and ATP levels.

All the nitrosoureas tested inhibit oxygen uptake (Clark electrode) by L1210 cells and cause some reduction in cellular ATP levels. The most marked inhibition of oxygen uptake is caused by CCNU while the least effective compounds are chlorozotocin (NSC 178248) and 1-(2-chloroethyl)-3-(cis-3-hydroxycyclohexyl)-1-nitrosourea (NSC 253945) (cis-3-hydroxy CCNU). CCNU caused a marked fall in cellular ATP levels; a parameter almost unaffected by similar treatments with chlorozotocin or cis-3-hydroxy CCNU. BCNU has an intermediate effect both on cell respiration and ATP levels.

The effect of the nitrosoureas on respiration and ATP levels appears related to their ability to form reactive isocyanates during their chemical breakdown. In cells which rely predominately on glycolysis for their ATP production, CCNU does not affect ATP levels suggesting that the mitochondrion is the target of the isocyanates.

2. Induction of single strand breaks in cellular DNA by various nitrosoureas.

The chemical breakdown products of the 1-(2-chloroethyl)-3-substituted nitrosoureas have varying chemical alkylating and carbanylating potentials. Those nitrosoureas which have the highest chemical alkylating potential (chlorozotocin and cis-3-hydroxy CCNU) have the lowest ability to induce single strand breaks in cellular DNA. Those nitrosoureas with low chemical alkylating potential but high carbanylating activity are more effective in the induction of single strand breaks in DNA. Isocyanates themselves at low concentrations cause significant damage to cellular DNA.

3. DNA repair.

An unusual feature of the DNA damage induced by CCNU is its apparent lack of repair. Present attempts to demonstrate repair DNA synthesis have proved inconclusive.

Our current knowledge does ot clearly identify the active antitumor agent related to the nitrosoureas. Both the alkylating and carbanylating properties appear to play a note in causing damage to DNA. Studies with a variety of nitrosoureas with differing properties should allow a clearer insight into those properties which are desirable and those undesirable in an effective nitrosourea anti-tumor agent.

Publications:

1. Hilton, J., Bowie, D.L., Gutin, P.H., Zito, D.M., and Walker, M.D.: DNA damage and repair in L1210 cells exposed to 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. Cancer Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06908-03 CL

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Intravenous Methyl-CCNU (NSC-95441) and PTG (NSC-122819) in the Treatment of Central Nervous System Neoplasms (Phase II)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael D. Walker, M.D.	Associate Director, BCRC	CL, NCI
Others:	Philip H. Gutin, M.D.	Clinical Associate	CL, NCI
	Ronald J. Cohen, M.D.	Clinical Associate	CL, NCI

COOPERATING UNITS (if any)
Medical Service

LAB/BRANCH
Clinical Branch

SECTION
Neurosurgery

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

SUMMARY OF WORK (200 words or less - underline keywords)

Methyl-CCNU and epipodophyllotoxin was evaluated as combination chemotherapy for efficacy in the treatment of malignant glioma in 23 patients. On preliminary analysis little efficacy was noted for the combination, although delayed cumulative bone marrow toxicity was noted. A combination of these agents in the treatment of malignant glioma does not seen of value.

Methyl-CCNU and epipodophyllotoxin (PTG) have been used singly with apparent efficacy in central nervous system tumors and, therefore, are an appropriate combination to evaluate. Patients with biopsy proven or clear cut evidence of intracerebral malignancy are given intravenous methyl-CCNU 130 mg/M^2 every 6-8 weeks and PTG 100 mg/M^2 weekly. Bone marrow toxicity is monitored closely with complete blood counts and platelet counts as well as SMA-12 profiles. Twenty-three patients entered this study at the time of disclosure. Preliminary analysis indicates little efficacy in this combination regimen. Delayed cumulative bone marrow toxicity was profound in a number of patients with severe thrombocytopenia (less than 50,000) and/or leukopenia (less than 2,000).

Patients on study will continue to be treated and observed although no new patients will be accrued to the study as it goes into final evaluation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06909-03 CL

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Phase II Evaluation of Intrathecal N,N',N"-Triethylenethiophosphoramide (Thiotepa-NSC-6396) in Meningeal Neoplasms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Michael D. Walker, M.D.	Associate Director, BCRC	CL, NCI
Others:	Philip H. Gutin, M.D.	Clinical Associate	CL, NCI
	John A. Levi, M.D.	Visiting Associate	CL, NCI
	Peter H. Wiernik, M.D.	Head, Clinical Branch	CL, NCI

COOPERATING UNITS (if any)

Medical Service, BCRC, NCI

LAB/BRANCH

Clinical Branch

SECTION

Neurosurgery

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

SUMMARY OF WORK (200 words or less - underline keywords)

Phase II evaluation of intrathecal thiotepa is being carried out in patients with meningeal leukemia and meningeal carcinomatosis. Thiotepa represents the third intrathecal chemotherapeutic agent available for the treatment of meningeal disease and approximately one-half of the patients showed a complete response with reduction in cerebral spinal fluid blast count. It is anticipated that additional patients will be accrued and this study completed during the course of the next year.

Project Description: Z01 CM 06909-03 CL

Phase I evaluation of intrathecal N,N',N''-Triethylenethiophosphoramide (Thiotepa-NSC-6396) was tolerated in doses of 10 mg/M^2 without CNS or bone marrow toxicity. Several partial remissions during Phase I study indicated that a Phase II evaluation should be carried out. Adult patients with demonstrated meningeal involvement from leukemia or carcinomatosis received 10 mg total dose every other day of thiotepa. Children below 10 years of age received 6 mg. Patients were evaluated for clinical response, clearing of the CSF, improvement in neurologic status and toxicity. Of 11 courses administered to 10 patients, 5 patients had a complete response with complete clearing of the CSF of blast cells. One patient achieved a partial response with a reduction of the CSF blast count by one half or more. Three patients failed to respond; two of whom had meningeal carcinomatosis. Two patients were considered non-evaluable.

Despite the pharmacologic evidence of the rapid disappearance of thiotepa from the CSF due to its small molecular weight and the lipid solubility, it appears as though intrathecal thiotepa does have some value in the treatment of meningeal disease. It is of note that the dose utilized (10 mg) is close to the systemic dose of thiotepa, thus extraordinarily high concentrations of thiotepa are being created in the CSF pathways. It is anticipated that an additional 10 patients will be placed on the study before final evaluation is made.

Publications:

1. Gutin, Philip H., Weiss, Howard, D., Wiernik, Peter H., Walker, Michael D.: Intrathecal N,N',N''-Triethylenethiophosphoramide (Thiotepa-NSC-6396) in the Treatment of Malignant Meningeal Disease, Phase I - II Study. Cancer (in press).

PREVIOUS PROJECT NUMBER (DO NOT use this space)	DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06911-04 LPH

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

In Vivo Metabolism of 3-Cyclohexyl Substituted 1-(2-chloroethyl)-1-Nitrosoureas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John Hilton, Ph.D.	Visiting Associate	LPH, NCI
Others:	Michael D. Walker, M.D.	Associate Director, BCRC	CL, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Neurosurgery

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0

SUMMARY OF WORK (200 words or less - underline keywords)

Stable chromogenic derivatives of the cyclohexyl moiety of hydroxylated CCNU have been prepared and separated by high pressure liquid chromatography.

Project Description: Z01 CM 06911-04 LPH

The cyclohexyl moiety of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) is rapidly hydroxylated in the rat and man to give a variety of monohydroxylated products. A total of six hydroxylation products are possible, five of which have been identified in the rat and three in man. The position of ring hydroxylation has been found to have profound effects on the chemical alkylating activity and carbanylating activity of these compounds (Z01 CM 06904-04 LPH).

The chemical instability of the nitrosoureas indicated that the preparation of stable derivatives of the hydroxylated metabolites would accelerate future investigations of the metabolism and distribution of these compounds. A variety of derivatives have been attempted. Only the mildest reaction conditions can be employed in order to maintain the nitroso function during the derivatisation. The most satisfactory procedure has been found to be esterification of the ring hydroxyl groups with acetic anhydride to form the stable acetoxycyclohexyl compounds. Breakdown of the acetylated nitrosourea in dilute alkali yields acetoxycyclohexylamines which can be readily derivatised on the amino group. Reaction of acetoxycyclohexylamines with alkali yields the corresponding 2,4-dinitrophenylaminocyclohexanol. These compounds are readily separated by reverse-phase high pressure liquid chromatography.

Publications:

1. Hilton, J., and Walker, M.D.: Hydroxylation of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. Biochem. Pharmacol. 24:2153-2158, 1975.

PROJECT NUMBER (Do NOT use this space)	DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06930-02 CL

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (50 characters or less)

Alkylation of DNA in the Rat Glioma Model by Intravenous CCNU

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Philip H. Gutin, M.D.	Clinical Associate	CL, NCI
Others:	John Hilton, Ph.D.	Visiting Associate	LPH, NCI
	Michael D. Walker	Associate Director, BCRC	CL, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

Neurosurgery

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

SUMMARY OF WORK (200 words or less - underline keywords)

Alkaline sucrose gradient analysis of DNA damage in the intracerebral rat glioma has showed definite DNA strand damage after the administration of therapeutic, non-toxic doses of CCNU. Compared to methyl methanesulfonate, a known alkylating agent, the mechanism of damage appears to be different. Additional studies to determine these mechanisms are being undertaken.

PHS-6040
(12-75)

Project Description: Z01 CM 06930-02 CL

It has been suggested that alkylation is the mode of action of CCNU, an effective agent in the treatment of recurrent malignant glioma. Using the rat intracerebral glioma model as an in vivo approach, the effects of DNA damage were determined using alkaline sucrose gradient analysis techniques. Preliminary studies determine CCNU toxicity in non-tumor bearing rats and the efficacy on rats bearing intracerebral gliosarcomas. Cell suspensions taken at various intervals were examined and the alkaline sucrose gradient analysis of DNA damage in the intracerebral right glioma showed definite DNA strand damage following the administration of therapeutic, but non-toxic doses of CCNU. Induced DNA damage was of a low level and anomalous sedimentation became a serious technical problem. Only very high doses of CCNU could induce the necessary damage to cause more conventional random sedimentation profiles.

A more quantitative method of looking at DNA damage utilizes the determination of DNA unwinding rates in alkali. Determination of the fraction of double stranded DNA after exposure to alkali was measured by its resistance to S-1 nuclease, a single strand specific exonuclease from Aspergillus oryzae. Using this method, it has been shown that CCNU at doses of 30 mg/kg causes DNA damage, i.e., single strand breaks, equivalent to methyl methanesulfonate at doses of 25 mg/kg. The damage caused by methyl methanesulfonate, however, was completedly repaired while the CCNU remained unrepaired at 16 hours. This suggests that CCNU versus methyl methanesulfonate, a known alkylating agent, produces its damage by a different mechanism. The data demonstrates that nitrosoureas caused DNA damage in rat brain tumor in vivo, however, whether this damage is caused by classical alkylation or other mechanisms remains to be determined. Additional mechanisms will be elucidated over the course of the ensuing year's work.

PROJECT NUMBER (do NOT use this space)

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NO.

Z01 CM 06931-02 CL

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (60 characters or less)

Intravenous CCNU Phase II Study in Colorectal Adenocarcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marshall A. Levine, M.D.	Clinical Associate	CL, NCI
Others:	Peter H. Wiernik, M.D.	Head, Clinical Branch	CL, NCI
	A. Collier Smyth, M.D.	Clinical Associate	CL, NCI

COOPERATING UNITS (if any)

Section of Medical Oncology, BCRC, NCI

LAB/BRANCH

Clinical Branch

SECTION

Neurosurgery

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

SUMMARY OF WORK (200 words or less - underline keywords)

A phase II evaluation of intravenous CCNU has been carried out in sixteen patients with advanced colorectal adenocarcinoma. CCNU was delivered in an emulfor and ethanol surfactant solution. Hematologic toxicities secondary to CCNU in addition to pulmonary toxicity including dyspnea and hypoxia were demonstrated. This study has been closed to the accrual of patients.

Project Description: Z01 CM 06931-02 CL

Colorectal adenocarcinoma has proven to be a neoplastic disease unresponsive to chemotherapy. Advance colorectal adenocarcinoma has a 2-6 month prognosis with less than 20% of the patients responding to any modality of systemic therapy. IV-CCNU showed some efficacy in several early patients with colorectal adenocarcinoma. Consequently, a formal phase II trial was undertaken.

130 mg/M^2 of CCNU was administered using a vehicle of 5% emulfor and 5% ethanol in D5W. Patients entered were generally untreated although several had received radiation therapy or 5-Fluorouracil chemotherapy. Patients were treated until definite tumor progression occurred.

The principal toxicities of IV-CCNU therapy were again noted in this phase II trial. Proven toxicity consisted of nausea and vomiting and diarrhea, chills and fever to 102°, and dyspnea and hypoxia to 55-60 mm. These were noted in many patients. The late toxicity consisted of thrombocytopenia occurring at 3-4 weeks and lasting 1-2 weeks, and leukopenia and anemia occurring at 4-5 weeks and lasting 1-2 weeks. Initially, dose limiting toxicity appeared to be 130 mg/M^2, but this was subsequently reduced to 100 mg/M^2 because of more severe hematologic toxicity than occurred in the phase I study of IV-CCNU.

Sixteen evaluable patients have been entered into this study of which only two have shown a partial remission. Remission duration was short and no specific advantages has been demonstrated for the utilization of IV-CCNU in the treatment of colorectal adenocarcinoma. In addition, the pulmonary toxicities noted presumably in relation to the vehicle have been encountered and, therefore, further accrual of patients to this study have been discontinued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06043-05 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (60 characters or less)

Automated Monitoring of Drug Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: *Clarence L. Fortner, Director, Clinical Research Pharmacy Service, CL-NCI*
James K. Hooper, Assistant Chief for Medical Computer Application, Health Services Research, USPHS Hospital

OTHER: *Michael D. Walker, Director, M.D., CL-NCI*
Richard Hsieh, Chief, Health Services Research, USPHS Hospital
William R. Grove, Clinical Pharmacist, CL-NCI
Peter H. Wiernik, Head, Medical Oncology Service, CL-NCI

COOPERATING UNITS (if any)

Medicine and Neurosurgical Service, BCRC;
Division of Health Services Research, Baltimore USPHS Hospital

LAB/BRANCH
Clinical Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	*.25*	*2*

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to provide efficient and rapid methods of automated collecting data concerning the clinical trials of experimental drugs.

The system of computer processing drug information provided accurate, periodic summaries of patient's drug therapy. Drug and patient identifiers are typed on the computer card and this data is read weekly by optical character recognition. The data is collated and a weekly summary is printed listing total dose and frequency of administration and omission of each drug. The cumulative report printed weekly lists cumulative doses by problem number for all drugs the patient has received. The problem number and statement according to the Weed's problem oriented health record system has been incorporated into this system.

PHS-6040

Project Description: *Z01 CM 06043-05 CL*

The objective of this project is to provide an efficient and rapid method of collecting data concerning the clinical trials of experimental drugs.

This is being studied by a computerized method of ordering and recording all drug therapy.

A system of processing drug administration information provides accurate, weekly summaries of each patient's complete drug therapy. The physician writes each drug order directly on a medication card, producing four copies: the medicine card copy, the pharmacy copy (for entry to a patient drug profile), and copies for medical records and automated analysis. The nurse charts medications given directly on the first copy of the drug form at the time of drug administration. This data is read weekly by optical character recognition. The data is collated and patient drug summaries are printed listing drug, dose, total dose, frequency of administration and a daily record of administration or omission of each drug. A monthly drug utilization report is also obtained as a by-product.

The problem number and statement according to the Weed's problem oriented health record system has been incorporated into the form which allows each drug to be listed by problem number on the weekly drug summary for each patient. Space on the drug form has also been allotted for the patients' height, weight, and body surface area. This gives the pharmacist enough data to recalculate all drug doses listed in research protocols. Not only does this recalculation protect the patient, but it also protects the research environment by insuring the accuracy of all doses administered. This system fulfills all the requirements of drug ordering, administration and recording, in addition to providing machine-readable data for computer input. The summaries are checked by the pharmacists, and become a permanent record of drug therapy both in the patient's chart and in the pharmacy. This system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies, analyzing drug effect, toxicity, and drug interaction. This system has reduced errors in drug administration and has increased staff awareness of the problems inherent in quality drug administration. A complete list of all drugs administered to the patient are recorded by problem numbers in a concise and orderly fashion in the patient chart. This list of all drugs administered has been used to obtain information about the cost of drug treatment for single medical problems. This cost may be found because of the development of a new cost model referred to as Problem Oriented List of Changes (POLC). This model is directly related to the Problem Oriented list of all drugs administered to each patient described above. The POLC may be used to determine the drug cost for single problems such as fever, granulocytopenia, etc. This system, as outlined, has provided a more comprehensive and efficient record of cancer chemotherapy drug trials.

Publications:

Esterhay, R.J., Vogel, V.G., Fortner, C.L., Sharpiro, H.M., Wiernik, P.H.: Cost analysis of leukemia treatment-A problem-oriented approach. CANCER. *37: 646-652, 1976.*

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06236-05 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Role of the Pharmacist in Obtaining a Patient's Drug and Allergy History

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Clarence L. Fortner	Director, Clinical Research Pharmacy Service, CL-NCI
OTHER:	William R. Grove	Clinical Pharmacist, CL-NCI
	John F. Bender	Clinical Pharmacist, CL-NCI

COOPERATING UNITS (if any)

Medicine and Neurosurgical Service, BCRC

LAB/BRANCH
Clinical Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

SUMMARY OF WORK (200 words or less - underline keywords)
To evaluate the feasibility of having a pharmacist interview all patients referred to the BCRC for the purpose of obtaining a drug and allergy history.

Pharmacists recorded drug and allergy histories have resulted in more complete drug and allergy history information. Some of the results obtained from these interviews are summarized: patients taking prescription drugs at the time of admission-75%, patients taking prescription drugs during the past year-90%, patients taking non-prescription drugs within a few weeks of admission-92%, and patients reporting a history of allergy to drugs, food, animals, clothing, iodine, and tape-31%. These interviews have provided the pharmacist with patient contact at the time of admission which has increased the pharmacist awareness of patient care. All medications brought to the hospital by the patient are obtained at time of the interview, and are stored in the pharmacy during the period of hospitalization. The pharmacists are responsible for placing all known allergic reactions information on the problem sheet of the patient's chart. At the time of completion of the interview, the original is placed in the history section of the patients hospital chart, and a duplicate is attached to the patients drug profile in the pharmacy. These interviews have demonstrated that the pharmacist can provide an additional useful service to the patient history.

The objective is to evaluate the feasibility of having a pharmacist interview all patients admitted to the Baltimore Cancer Research Center for the purpose of obtaining a complete drug and allergy history.

The pharmacist questions each patient admitted to the Medicine and Neurosurgical Services, BCRC, concerning current and past medication usage and history of allergy.

A written record of this interview is placed in the history section of the patient's chart and in pharmacy's drug profile for each patient.

Pharmacists recorded drug and allergy histories have resulted in more complete drug and allergy history information. Some of the results obtained from these interviews are summarized: patients taking prescription drugs at the time of admission-75%, patients taking prescription drugs during the past year-90%, patients taking non-prescription drugs within a few weeks of admission-92%, and patients reporting a history of allergy to drugs, food, animals, clothing, tape and iodine-31%. The interview includes determining all of the over the counter (OTC) drugs that the patients have received without professional supervision. This information is extremely valuable at the time the patient is discharged from the hospital. The pharmacist is able to utilize this information by advising the patient of any drug-drug interactions, etc. between the OTC drug and drugs being prescribed by the physician upon discharge. These interviews have provided the pharmacist with patient contact at the time of admission which has increased the pharmacist awareness of patient care. All medications brought to the hospital by the patient are obtained at time of the interview, and are stored in the pharmacy during the hospitalized period. The pharmacists are responsible for placing all known allergic reaction information on the front of the patients drug Kardex, and on the problem sheet in the patient's chart. At the time of completion of the interview, the original is placed in the history section of the patients hospital chart and a duplicate is attached to the patients drug profile in the pharmacy. If the pharmacists feel that some of the information should be brought to the physicians attention immediately, he also communicates verbally to the physicians. Duplicates of the pharmacist acquired inpatient histories are placed in the outpatient drug profile record. This information may then be utilized as the patient returns to the outpatient department for new or additional drugs. This has been a useful tool and we plan to initiate within the next year to obtain drug and allergy histories for all new outpatients. These interviews have demonstrated that the pharmacist is interested in patient care, and that he can provide an additional useful service. The drug and allergy interview by the pharmacist has become an integral part of the clinical pharmacy program. The present study will be continued and the outpatient portion will be expanded.

PUBLICATIONS:

Cradock, J. and Fortner, C.L.: The Pharmacist-acquired Medication History. Clinical Pharmacy Sourcebook, Publishing Sciences Group, Inc., 1976, Acton, Massachusetts.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06237-06 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Development of a Clinical Research Pharmacy Service

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Director, Clinical Research Pharmacy Service,CL-NCI

OTHER: William R. Grove, Clinical Pharmacist, CL-NCI

Robert R. Foreman, Clinical Pharmacist,CL-NCI

COOPERATING UNITS (if any)

Medicine and Neurosurgical Service, BCRC

LAB/BRANCH
Clinical Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.50	*1.50*	*1.0*

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to determine the role of the pharmacist in the patient care area, and as a member of the health care team actively contributing to the patient's care.

The pharmacist has proven that he can be patient oriented rather than product-oriented. The pharmacist utilizing new methods has become a valuable member of the health care team and in contributing directly to the patient care. The following has been accomplished: improving drug prescribing practices by promoting the rational and safe use of drugs, preventing potential drug-drug interactions, detecting and preventing adverse drug reactions, detecting drug induced laboratory test abnormalities, detecting possible drug induced disease, providing drug information, continuing education for various members of the health care team, counseling the patient on proper use of drugs, and detecting and preventing intravenous admixture incompatibilities.

PHS-6040
(12-75)

Project Description: *Z01 CM 06237-06 CL*

The objective of this study is to determine the role of the pharmacist in the patient care area, and as a member of the health care team actively contributing to the patient's care. Patient education has been added to this study. The objective of this education will be to increase compliance and reduce problems for outpatients receiving drugs.

The pharmacist is located in the midst of the patient care area in order to allow him to investigate new concepts, approaches, and research opportunities to aid in the patient care. The pharmacist attends morning and evening ward rounds, reviews charts, interviews patients and is familiar with the patients' condition. Therefore he can intelligently discuss therapy problems with the patient's physician. With this information, he can also provide the staff with drug information.

An integral part of this service is the therapy surveillance system. The nucleus of the drug surveillance system is the individual patient drug profile. The pharmacist receives a direct copy of the physician drug order and transcribes it to the patient drug profile. The drug profile includes the patient name, primary and secondary problems, physician name, known allergies or idiosyncrasies and all drug orders. The pharmacist reviews each new drug order for completeness and recalculates all protocol drug dosages then reviews the drug profile against the new order for drug interactions, laboratory problems, dietary problems and possible adverse drug reactions. The drugs are then dispensed thru a unit dose system which provides accurate records. All returned doses to the pharmacy are investigated for reason of non-compliance. The monitoring system involves evaluating the necessity of continued medication orders.

The pharmacist has showed that he can be patient oriented rather than product oriented. The pharmacist utilizing the above methods has become a valuable member of the health care team and contributes directly to the patient care. The following has been accomplished: improving drug prescribing practices by promoting the safe and rational use of drugs, preventing potential drug-drug interactions, detecting and preventing adverse drug reactions, detecting drug induced laboratory test abnormalities, detecting possible drug-induced diseases, providing drug information, counseling the patient on proper use of drugs, and detecting and preventing intravenous admixture incompatibilities. This has reduced drug exposure to the patient, decreased nursing and pharmacy time spent administering and preparing drugs and reduced cost.

A higher quality of drug prescribing and reduced medication errors has contributed toward better patient care and has enhanced the quality of the research data.

Drug information is becoming a major by-product of locating the pharmacist in the patient care area. This information is being requested by all members of the health care team. Continuing education is also involved in this process of providing drug information. The impact of this service will continue to be studied by the audit process.

Present studies will be continued and additional methods to improve patient care, will be further researched.

PUBLICATIONS:

Grove, W.R., Fortner, C.L.: Carcinoma of the Breast. Clinical Pharmacy and Therapeutics edited by E. Herfindal and J. Hirschman, Baltimore-Williams and Wilkins, 483-495, 1975.

Christian,D., Cornelis, W., Fortner, C.L.: Leukemias. Clinical Pharmacy and Therapeutics edited by E. Herfindal and J. Hirschman, Baltimore-Williams and Wilkins, 459-471, 1975.

Cornelis, W., Christian, D., Fortner, C.L.: Hodgkin's Disease. Clinical Pharmacy and Therapeutics edited by E. Herfindal and J. Hirschman, Baltimore-Williams and Wilkins, 471-483, 1975.

Wiernik, P.H., Schimpff, S.C., Schiffer, C.A., Lichtenfeld, J.L., Aisner, J., O'Connell, M.J., Fortner, C.L.: Randomized Clinical Comparison of Daunorubicin (NSC-82151) Alone with a combination of Daunorubicin, Cytosine Arabinoside (NSC-63878), 6-Thioguanine (NSC-752), and Pyrimethamine (NSC-3061) for the Treatment of acute nonlymphocytic Leukemia. Cancer Treatment Reports, Vol. 60, No. 1., Jan. 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06912-02 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Antineoplastic Drug Displays for the Computerized Problem-Oriented Medical Information System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William R. Grove, Clinical Pharmacist, CL-NCI
Clarence L. Fortner, Director, Clinical Research Pharm. Service, CL-NCI

OTHER: Paul Chang, M.D., Senior Investigator Med. Oncology Service, CL-NCI
Robert Esterhay, M.D., Senior Investigator, CL-NCI
George Nelson, M.D., PROMIS Laboratory, Dept. Med., University of Vermont, Burlington, Vermont
Peter Wiernik, M.D., Head, Med. Oncology Service, CL-NCI

COOPERATING UNITS (if any)

Medicine and Neurosurgical Services, BCRC
PROMIS Laboratory, Dept. of Medicine, University of Vermont

LAB/BRANCH
Clinical Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.16	.16	0

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to prepare referenced summarized pharmacologic information which is readily retrievable at the touch-sensitive cathode ray screen terminal located at the BCRC nursing station.

Current literature is reviewed to obtain accurate and complete drug information on antineoplastic agents. The standardized format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS, TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, AND METABOLISM AND EXCRETION. Reference codes are included with all pages of information, which may be further explored by contacting either the Clinical Research Pharmacy Service of the BCRC or the PROMIS Laboratory, University of Vermont. After completion, display sequences are sent to the PROMIS laboratory to be audited for accuracy, completeness, and correctness of clinical judgement before being entered into the computerized system.

The completed antineoplastic drug displays provide ready access to referenced summaries of the current medical literature. They form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS system.

Project Description:

The objectives of this project are to prepare referenced summarized pharmacologic information which is readily retrievable at the touch-sensitive cathode ray screen terminal located at the BCRC nursing station. These displays are to be complete, concise and accurate summaries of currently-available literature. Additional antineoplastic drugs are added to the computerized drug displays already on file with the PROMIS system, Burlington, Vermont.

Current literature is reviewed to obtain accurate and complete drug information on antineoplastic agents. This information is then organized to a specific format to conform with the over 20,000 displays currently included in the PROMIS system. The standardized format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS, TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, AND METABOLISM AND EXCRETION. Reference codes are included with all pages of information, which may be further explored by contacting either the Clinical Research Pharmacy Service of the BCRC or the PROMIS Laboratory, University of Vermont. After completion, display sequences are sent to the PROMIS laboratory to be audited for accuracy, completeness, and correctness of clinical judgement before being entered into the computerized system. Previously entered data may be updated as necessary by providing the new information and references to the PROMIS laboratory for incorporation.

The completed antineoplastic drug displays provide ready access to referenced summaries of the current medical literature. They form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06937-01 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

A Patient Oriented Outpatient Drug Distribution Service

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Director, Clinical Research Pharm. Service, CL-NCI
OTHER: William R. Grove, Clinical Pharmacist, CL-NCI

COOPERATING UNITS (if any)

Medicine and Neurosurgical Services, BCRC

LAB/BRANCH
Clinical Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	*1*	*0*

SUMMARY OF WORK (200 words or less - underline keywords) *The objectives of this service are to dispense prescriptions to outpatients and patients being discharged from the hospital, maintain an individual outpatient drug profile, and provide necessary drug information and drug administration instructions to outpatients. When the patient brings the prescription to the pharmacy, the pharmacist reviews the order for completeness and against any previously prescribed drugs for drug-drug interactions, and any known allergies.*

The profile may be used in several additional methods. Any changes in dosages, particularly chronic medications, are verified with the physician. Also, when a patient is admitted, the outpatient profile serves as a method by which the pharmacist may verify that necessary medications are ordered for that inpatient. Similarly, when a patient is discharged, his inpatient medication orders are checked against his outpatient prescriptions to ensure all necessary medications are being continued as an outpatient.

The prescription will then be prepared in the traditional manner. The patient will be advised, at the time of dispensing, of common side effects and specific administration instructions. Patient compliance is stressed by the pharmacist. When follow-up instructions indicate poor patient compliance, the pharmacist promptly notifies the physician.

PHS-6040
(12-75)

Project Description:

The objectives of this service are to dispense prescriptions to outpatients and patients being discharged from the hospital, maintain an individual outpatient drug profile, and provide necessary drug information and drug administration instructions to outpatients. These studies will include a method of best determining drug-drug interactions, drug-food interactions and known patient allergies prior to dispensing the drug to the patient. New patients will be interviewed concerning known allergies and sensitivities. Any relevant information found is entered onto the individual drug profile and relayed to the patient's physician. When the patient brings the prescription to the pharmacy, the pharmacist reviews the order for completeness and against any previously prescribed drugs for drug-drug interactions, and any known allergies.

The profile may be used in several additional methods. Any changes in dosages, particularly chronic medications, are verified with the physician. Several inadvertant changes have been corrected by this method. The profile is useful in providing ready answers to questions from physicians concerning previous outpatient medications. Also, when a patient is admitted, the outpatient profile serves as a method by which the pharmacist may verify that necessary medications are ordered for that inpatient. Similarly, when a patient is discharged, his inpatient medication orders are checked against his outpatient prescriptions to ensure all necessary medications are being continued as an outpatient.

The prescription will then be prepared in the traditional manner. The patient will be advised, at the time of dispensing, of common side effects and specific administration instructions. Patient compliance is stressed by the pharmacist. In addition, relevant drug-drug interactions and drug-dietary interactions will be discussed, as indicated. When follow-up instructions indicate poor patient compliance, the pharmacist promptly notifies the physician. Results indicate proper use of the individual drug outpatient profile increases proper utilization of outpatient prescriptions and increases patient compliance.

MATHEMATICAL SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06238-05 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Development of a Unit Dose Service and the Preparation of Parenteral Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clarence L. Fortner, Director, Clinical Research Pharmacy Service, CL-NCI
OTHER: William R. Grove, Clinical Pharmacist, CL-NCI

COOPERATING UNITS (if any)

Medicine and Neurosurgical Service, BCRC

LAB/BRANCH
Clinical Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	2	2

SUMMARY OF WORK (200 words or less - underline keywords)

To improve patient care through a new drug delivery system and to provide more accurate information regarding the disposition of drugs.

The two systems, unit-dose and I.V. admixture, have proven to be advantageous over previous drug distribution systems. They have placed the preparation and distribution in the hands of the pharmacist. These systems have accomplished the following: reduced medication errors, reduced medication cost, enhanced the pharmacist as a drug information consultant by relating him more closely to the patient, reduced the possiblility of contamination of the drug, permits nurses' to use time more effectively in direct patient care and in general allows the hospital to demonstrate adequate control over the distribution of all drugs. In addition, the pharmacist has instituted record systems that have improved retrieval of the patient's drug records. Lastly, a sterile fat emulsion preparation had been used in over 300 intravenous injections for the administration of an aqueous insoluble drug.

The objective is to improve patient care through a new drug delivery system and to provide more accurate information regarding the disposition of drugs.

A unit dose of medicine is a single dose of medicine packaged for an individual patient. This single dose package is labeled with a name, strength, and control number of the drug, and it is ready to be administered to the patient. These drugs are ordered by the physician and they are placed in the individual patients drawer of a medication cart by the pharmacist. This cart is designed so that there is a single drawer for each patient's drug. All of the parenteral products are prepared aseptically in a laminar air flow environment, and are dispensed in a clearly labeled ready to use form. Parenteral products are prepared for not more than a 24 hour period to ensure full potency. The parenteral drugs are not placed in this cart. They are carried directly to a refrigerator in the nursing unit. Control procedures for checking the composition of the product prepared, and for monitoring bacterial and particulate matter contamination of these preparations have been instituted. Pertinent information regarding the drugs dispensed is recorded on specially designed forms that are filed according to the patient's name. This information is also recorded by drug and lot number for each investigational agent.

The two systems, unit-dose and I.V. admixture, have proven to be advantageous over the old ward stock system of drug distribution. They have placed the preparation and distribution in the hands of the pharmacist. These systems have accomplished the following: reduced medication errors, reduced medication cost, enhanced the pharmacist as a drug information consultant by relating him more closely to the patient, reduced the possibility of contamination of the drug, permits nurses to use time more effectively in direct control over the distribution of all drugs. In addition, the pharmacist has instituted record systems that have improved retrieval of the patient's drug record. Physicians utilize these records daily in ordering medications for patients and have found them to be a useful and accurate source of information.

During the past year the pharmacy service has dispensed approximately 207,768 oral doses of medication to inpatients, 31,381 I.V. admixtures, 10,361 doses of parenteral investigational cancer chemotherapeutic agents, and 3,492 sundry sterile preparations.

By reducing medication errors, and improving the accuracy of the records of disposition of cancer chemotherapeutic agents and the other unit dose drugs the quality of patients care has increased. These delivery systems have been integrated with the development of the Clinical Research Pharmacy Service which is providing the patients with better care. Thus, the research drug data will be improved.

Clinical studies have been completed utilizing an acceptable sterile fat emulsion preparation for the intravenous administration of an aqueous insoluble drug. This fat emulsion has been sucessfully used without side effects in over 300 intravenous administrations.

Publications:

Fortner, C.L., Grove, W.R., Bowie, D., Walker, M.D.: Fat emulsion vehicle for intravenous administration of an aqueous insoluble drug. American J Hosp Pharm. 32: 582-584, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06943 CL

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Audit of the Outpatient Drug Profile

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATOR AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED IN THE PROJECT

PI:	William R. Grove	Clinical Pharmacist	CL-NCI
Other:	Clarence L. Fortner	Director	CL-NCI

COOPERATING UNITS (if any)

Medicine and Neurosurgical, BCRC

LAB/BRANCH
Clinical Branch

SECTION
Clinical Research Pharmacy Service

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.10	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to promote optimal patient care through appropriate utilization of the outpatient drug profile. The process audited is the pharmacists' completion and utilization of the outpatient drug profile. Audit criteria were defined as audit standards, audit method, audit interval, audit form, and feedback system.

An initial audit of sixteen randomly selected outpatient profiles was performed. Several observations were made, including: Prescription entries are being completed appropriately by the pharmacists; Protocol chemotherapy doses were verified as correct; Drug and allergy histories conducted by the pharmacist were not present in every profile; Problem numbers and statements have not been completed on all prescriptions by the physicians.

Several recommendations were made to the members of the Clinical Research Pharmacy Staff based on the results of this audit to improve the utilization of the outpatient drug profile.

This study will continue with additional audits to be performed periodically. The results of subsequent audits will be compared against this initial one to determine if improvement has occurred.

Project Description: Z01 CM 6943-01 CL

The purpose of this study is to promote optimal patient care through appropriate utilization of the outpatient drug profile. The process audited is the pharmacists' completion and utilization of the outpatient drug profile. Audit criteria were defined as audit standards, audit method, audit interval, audit form, and feedback system.

An initial audit of sixteen randomly selected outpatient profiles was performed. Several observations were made, including: Prescription entries are being completed appropriately by the pharmacists; Protocol chemotherapy doses were verified as correct; Drug and allergy histories conducted by the pharmacist were not present in every profile; Problem numbers and statements have not been completed on all prescriptions by the physicians.

Several recommendations were made to the members of the Clinical Research Pharmacy Service Staff based on the results of this audit to improve the utilization of the outpatient drug profile.

This study will continue with additional audits to be performed periodically. The results of subsequent audits will be compared against this initial one to determine if improvement has occurred.

SUMMARY REPORT

Laboratory of Pharmacology, Baltimore Cancer Research Center, DCT, NCI
July 1, 1975 through June 30, 1976

Within the Enzymology and Drug Metabolism Section, concentration has been on gaining a greater understanding of the physiological role of polyamines in regulating cell metabolism. Previous work from the Section and elsewhere has shown a strong correlation between the presence of polyamines and RNA synthesis. Since the concentration of RNA within the cell is not only a function of the amount synthesized, but also of the amount degraded, examination of the relationship between these substances and degradative enzymes (the ribonucleases) was undertaken.

Of particular interest in this regard has been the observation that polyamines can cause not only an enhancement of RNase activity, but can also reverse the inhibition of RNase activity caused by certain polynucleotides. Thus, polyguanylic and polyadenylic acids, which act as competitive inhibitors of RNase activity, are dissociated from the enzyme surface by spermidine. The significance of this is that it may help explain why the RNA in an RNA virus or in a messenger RNA is not readily degraded in mammalian cells. Both these RNAs have poly A at their 3' OH termini which can inhibit RNase activity. At the appropriate time the concentration of polyamine can rise causing reversal of the inhibition and subsequent degradation of the messenger RNA.

In view of the earlier findings that polyamines in human urine may have diagnostic importance in detecting neoplasia in man, the isolation of a protein (human plasma ribonuclease) from human plasma that can bind the polyamines is of considerable significance. The enzyme, although found in high concentrations in multiple myeloma, is related to that disease only in the sense that accompanying multiple myeloma is severe kidney disease. The enzyme concentration was found elevated in severe kidney malfunction unrelated to multiple myeloma.

Ongoing studies within the Section on specific ribonucleases and deoxyribonucleases have been of importance in the elucidation of the termini of an RNA isolated from AKR murine leukemia virus. Thus, uridylic acid has been found at the 3' terminus, whereas adenylic acid is at the 5' terminus of the long chain polymer. Currently, studies are being undertaken to examine the polarity of the viral RNA, particularly with respect to the section of the polymer coding for reverse transcriptase.

In collaboration with the Oncology Service of the Baltimore City Hospitals, studies on the use of polyamine levels in human urine as an indicator of the presence of neoplasia are ongoing. Thus far from the results obtained with patients undergoing extensive chemotherapy, what appears to be of particular significance in evaluating the disease's state is the concentration of spermidine.

An additional finding of interest is that a number of ribonucleases are bound to DNA structures called plasmids contained within a microorganism. These plasmids are made of circular duplex DNA and carefully modulate ribonuclease

activity in the cell. This would suggest that no nucleolytic activity goes unregulated in a living organism. In mammalian cells it is of interest that nucleolytic enzymes are also carefully controlled. We find that in the pancreas, for example, the enzyme is bound to a polyribonucleotide which inhibits ribonuclease activity. The relation between this careful modulation and disease states is currently being investigated.

The Section of Immunology and Cell Biology has continued work utilizing immunologically induced in vitro lymphocyte responsiveness as a model of human lymphocyte reactivity to non-self. Important advances have been made which relate both generally to the evaluation of host immune responsiveness, the viral infectious disease area and more specifically, to applications of this assay for evaluation of lymphocyte reactivity to putative oncogenic viruses. This laboratory has presented evidence that purified (banded) rubeola virus is not capable of eliciting responsiveness in this system but that responsiveness can be observed when the cell membrane preparation from which the virus is purified is used as antigen. This finding is of importance since it points to the need for membrane associated antigen as the recognition template (either viral or cell) in order for lymphocyte responsiveness to be achieved. Application of this observation to other viruses has indicated that a viral infected cell membrane preparation is generally the best antigenic source to determine previous host exposure to virus in the lymphocyte blastogenesis system. Studies in this laboratory have also focused on defining the potential and mechanism of action of various immune adjuvants in the prevention and treatment of neoplasia. The effectiveness of polyadenylic polyuridylic acid in the prevention of AKR leukemia has been re-explored and an age dependent effect was noted. It has been defined that the beneficial effect of polyadenylic polyuridylic acid in prolonging survival and preventing leukemia in the AKR mouse was dependent upon giving this agent at an early age rather than late. Additional work has explored the generally accepted concept that immunotherapy is most effective when tumor burden is at its lowest point. It has been found that immunotherapy initiated at a point of low tumor burden may result in some situations in decreased survival and that increased survival is observed if treatment is delayed. This work is of importance because it brings to the forefront the concept that tumor antigenic concentration must be sufficient for the host to respond successfully to tumor. Because of immune adjuvant work suggesting that abnormal ribonuclease activity existed in mice with neoplasia a series of studies have been carried out reflecting that abnormal nucleolytic activity is capable of predicting the development of tumor in high incidence tumor strain mice. Similarly, elevation of nuclease activity was found in the serum of white cells of mice with transplantable leukemia, decreased depression of nuclease activity in the white cells of guinea pigs bearing L2C leukemia and an elevation of serum nuclease activity in patients with neoplasia.

Studies concerning the interaction of tumor specific antibody and tumor and normal cells has indicated that the presence of an active homologous complement source capable of mediating tumor specific antibody lysis of tumor cells is also capable of mediating significant cross reactivity to antigenic determinants on normal cells. This cross reactivity is not observed when this same antibody is absorbed in the absence of an active complement source.

Research from the Biochemistry Section has clarified the mechanism by which anthracyclines undergo reductive glycosidic cleavage and has shown the remarkable dependence of the reductive glycosidase on anaerobic conditions. Because of the widespread occurrence of this type of enzyme, its importance in intermediary metabolism and as a protective metabolism are appreciated. A relationship of aldo-keto reductases (ex. in the reduction of adriamycin and daunorubicin and oxisuran) throughout vertebrate drug metabolism has been made. Our data in the biotransformation sphere have been useful to the study of clinical pharmacology. Human urinary metabolites of adriamycin have been isolated and identified. Human metabolism of adriamycin involves carbonyl reduction, reductive glycosidic cleavage, hydrolytic glycosidic cleavage, 0-demethylation, 0-sulfation, and -glucuronidation. Carbonyl reduction was a major enzymatic conversion occurring in the human. Other techniques and methodologies are being developed for further studies of these pharmacologic agents.

In our studies on control mechanisms affecting cellular growth and differentiation we have accomplished a task plaguing cell biologists and biochemists for years, and we have unraveled the structure of the phytohemagglutinin (PHA) isolectins. We have isolated five isolectins with graded activities from the red kidney bean. This procedure has allowed us to establish the structure of the isolectins as five proteins each consisting of tetramers of two different classes of subunits,and L-subunit and an E-subunit, such that the five proteins are identified as L4, L3E1, L2E2, L1E3, and E4. The elucidation of these isolectins and the demonstration of their specific biological activities are a cornerstone to the many studies where phytohemagglutinin (PHA) is and was used. In a study to elucidate the characteristics of Hairy cell leukemia, we have utilized the purified isolectins to characterize the biochemical activities of these cells. Hairy cells were studied ultrastructurally, cytochemically, and functionally. From the data it would appear that the Hairy cells appear to be a heterogenous population with characteristics of both mononuclear cells and lymphocytes. Continuing studies on myelomonocytic leukemia animal model (WEHI-3 tumor cells) have established the transplacental passage of the WEHI-3 tumor viruses.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06012-06 LPH

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Biochemical Pharmacology of Antineoplastic and Other Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Head, Biochem. Sect.	LPH, NCI
OTHER:	R. L. Felsted	Senior Staff Fellow	LPH, NCI
	H. Kubo	Visiting Fellow	LPH, NCI
	N. Ahmed	Visiting Fellow	LPH, NCI
	M. Kaber	Lab. Tech.	LPH, NCI
	M. Gee	Chemist	LPH, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Pharmacology

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

SUMMARY OF WORK (200 words or less - underline keywords)

The mammalian biotransformation of the anthracycline glycosides and the immunosuppressive oxisuran illustrate two unappreciated phenomena in mammalian biochemistry. The microsomal reductive glycosidase is an enzyme which is controlled by the oxygen content of the tissue. Such enzymes are likely important under hypoxic conditions. The aldo-keto reductases exemplified by rat liver aldehyde reductase and oxisuran reductase are drug metabolizing enzymes of the cellular cytoplasm. These reductases have very similar characteristics and may be related.

Project Description:
To study the biochemical pharmacology of antineoplastic and other drugs and to provide a scientific foundation for their usage. To resolve the metabolic pathways and the interaction of these drugs and their metabolites with tissue components in vitro and with organized physiological systems in vivo.

Rat liver microsomes contain a phenobarbital inducible, NADPH dependent, reductive glycosidase capable of cleaving several anthracycline antibiotics including adriamycin, daunorubicin, and carminomycin to deoxyaglycones. Molecular oxygen reversibly inhibits the microsomal enzyme greater than 95% at 20% oxygen partial pressure. Carbon monoxide, SKF 525A and sulfhydryl reagents do not inhibit the reaction. Since the intact glycoside is necessary for conversion to the deoxyaglycone, a concerted reaction mechanism is proposed. The reductive glycosidase activity is also present in other tissues. Sensitivity of this enzyme to molecular oxygen suggests a possible regulatory role for the enzyme in vivo.

Rat liver aldehyde reductase is a soluble constitutive enzyme having the ability to catalyze the reduction of several natural aldehydes. On the basis of apparent Km, Vmax, and relative pseudo-second order rate constants (kcat/Km) 4-carboxybenzaldehyde is the best substrate and other xenobiotic aldehydes are excellent substrates for the enzyme. Low or negligible activity is seen for short chain aliphatic aldehydes. The only significant ketone substrate is the antibiotic daunorubicin. Inhibitors of the classical alcohol dehydrogenase do not inhibit the aldehyde reductase. These and other properties suggest that this enzyme belongs to a class of reductases ubiquitously distributed among animal tissues. We therefore recommend that this enzyme be classified as a general alcohol:$NADP^+$ oxidoreductase (E.C.1.1.1.2).

The immunosuppressive drug 2-(methylsulfinyl) acetyl pyridine, oxisuran, is reduced extensively in vivo. We investigated the biochemical mechanism of oxisuran reduction in rabbit tissue preparations. The highest enzymatic activity occurred in liver followed by kidney, brain, intestine, lung, and blood. Subcellular localization revealed most activity was in soluble cell fractions but mitochondria also contained activity. The soluble liver enzyme showed NADPH dependency and an optimal pH of 6.0 for oxisuran reduction. Isoelectric focusing of the soluble liver fraction resolved three oxisuran reductase activities. Oxisuranol was identified and verified by chromatographic and mass spectrometric analysis.

The elucidation of the biotransformations of antineoplastic agents may be useful in understanding the pharmacodynamics and toxicity of these agents. Additionally, understanding the metabolic machinery can be useful in manipulating the affects of the drugs.

To study the nature and characteristics of the microsomal reductive glycosidase and the aldo-keto reductases.

Publications:
Takanashi, S. and Bachur, N.R.: Daunorubicin metabolites in human urine. J. Pharmacol. Exp. Therap. 195: 41-49, 1975.
Bachur, N.R.: Adriamycin pharmacology. Cancer Chemotherap. Rep. 6: 153-158, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06014-06 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Cellular Control Mechanisms Affecting Cell Growth and Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Head, Biochem. Sect.	LPH, NCI
OTHER:	M. J. Egorin	Clinical Associate	LPH, NCI
	R. L. Felsted	Senior Staff Fellow	LPH, NCI
	F. T. Sanel	Research Biologist	LPH, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Biochemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	0

SUMMARY OF WORK (200 words or less - underline keywords)

The isolation of substances from plant and animal sources which effect the growth and differentiation of normal and malignant human cells is paramount in the study of the processes which control cellular activities. By studying these purified materials as biochemical tools, we may be able to understand the biochemical mechanisms controlling cell proliferation and differentiation. Also, by investigating points where control of metabolic pathways are possible, we may find chemical agents to control cellular growth, differentiation, and proliferation. We have clarified the problem of the specificity of isolectins from Phaseolus vulgaris phytohemagglutinin in this phase of the studies.

PHS-6040
(12-75)

Project Description:
To study the control mechanisms which affect cellular growth and differentiation. These studies are aimed at elucidating control principles effecting all levels of cellular machinery as well as various levels of physiological controls between differentiated and nondifferentiated tissues and between malignant and normal tissues. To study the effects of control changes on physiologic processes.

Five isolectins of different biological activity were isolated from red kidney bean. The two-step isolation procedure easily and economically yields up to 50 mg of each protein. First, a mixture of the five lectins is obtained by affinity chromatography of a saline-bean extract on porcine thyroglobulin-conjugated Sepharose (Matsumoto and Osawa). Then five proteins (L4, E1L3, E2L2, E3L1, and E4) are separated on SP-Sephadex with sequential elution by a shallow gradient of increasing ionic strength. Each protein electrophoreses as a single homogeneous band of increasing mobility on polyacrylamide gel. E1L3 through E4 have increasing red cell agglutinating activity; L4 is inactive. L4 through E4 show decreasing ability to stimulate human peripheral blood lymphocytes in culture. Guanidine-urea dissociated isolectins were subjected to simultaneous isoelectric focusing on sheets of polyacrylamide. The protein stained patterns are consistent with the hypothesis that the isolectins are tetramers of two different classes of subunits. L4 is a tetramer of one subunit which is lymphocyte stimulating. E4 is a tetramer of the other subunit, which is erythrocyte agglutinating. E1L3, E2L2, and E3L1 differ only in the ratio of these two subunits.

We will continue our studies on the control mechanisms which affect cellular growth and differentiation, and attempt to elucidate control principles affecting all levels of cellular machinery. Specifically, we hope to purify hepatic stimulator substance and to describe its physiological action in the normal animal. We hope to elucidate the mechanism of action of the isomitogens on cell membranes. Thirdly, we will continue our search for materials characteristic of altered metabolic pathways, which may be used to quantify malignant cells in vivo.

Publication:
Felsted, R.L., Leavitt, R.D., and Bachur, N.R.: Purification of the phytohemagglutinin family of proteins from red kidney beans (Phaseolus vulgaris) by affinity chromatography. Biochim. Biophys. Acta 405: 72-81, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06016-06-LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Pharmacodynamics of Cancer Chemotherapeutic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Head, Biochem. Sect.	LPH, NCI
OTHER:	S. D. Reich	Clinical Associate	LPH, NCI
	S. Gordon	Lab. Tech.	LPH, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Biochemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of the structure activity relationships of the anthracycline antibiotics have centered on alterations at the C-9 position and substitutions on the amino group. These have indicated the importance of polarity of the agent for membrane transport as well as change of the agent for nucleic acid interferences.

PHS-6040
(12-75)

Project Description:
Alterations in the C-9 side chain of the anthracycline antibiotics, adriamycin and daunorubicin, have a profound effect on antibiotic uptake and accumulation by cultured L1210 cells. The degree of inhibition of DNA and RNA metabolism in the L1210 cells is directly related to the cellular uptake and accumulation of the drug analogs. Polar drug metabolites, daunorubicinol and adriamycinol, retain inhibitory activity against nucleic acid metabolism but have a decreased membrane binding and permeability. The chemically modified C-9 analog Rubidazone although sterically enlarged at C-9 is transported very well. Cellular uptake and accumulation of the C-9 analogs are inversely related to drug polarity. We propose that the polarity of the anthracycline analogs contributes heavily to the differences in therapeutic index and in vivo activity through fundamental effects on membrane permeability, metabolism, and macromolecular binding.

The antitumor antibiotics daunorubicin and adriamycin elicit a differential dose response from DNA polymerases purified from E. coli infected with mutator (L56), wild-type (T4D) and antimutator (L141) strains of bacteriophage T4. In a 0.02 - 0.06 mM range of anthracycline concentrations, stimulation in nucleotide incorporation is observed for L56, while T4D and L141 polymerases are inhibited by more than 35% and 85% respectively. Under similar assay conditions, but in the absence of deoxynucleoside triphosphates, the polymerase associated 3'-exonuclease activities are inhibited differentially with L56 now exhibiting greater and L141 least sensitivity. Similar inhibition patterns are observed for polymerase assayed in the presence of daunorubicin derivatives modified by attachment of various groups directly to the anthracycline ring. However, when the amino group attached to the sugar moiety is blocked (N-acetyl daunorubicin), a significant diminution of the differential dose response and a complete loss of L56 stimulation is observed. The potential value of the anthracyclines as probes to "tune" exonuclease to polymerase ratios will be discussed in terms of elucidating detailed molecular processes of error induction and correction.

To investigate the biochemical and physiological effects of antineoplastic drugs and their mechanism of action. To correlate the effects and activities of the drugs with their chemical structures.

Understanding the structure activity relationships of drugs is useful in planning new improved agents. Further explanations through study of the pharmacodynamics can help clarify problems in chemotherapy.

Through the continued investigation of the pharmacodynamics of drugs we may be able to determine why these agents possess differences in their therapeutic index and spectrum of action.

Publication:
Bachur, N.R., Reiter, W., and Arena, E. Cardiac uptake of adriamycin not affected by strophanthin G. Cancer Chemotherap. Rep. 59: 765-766, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06241-06 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Clinical Pharmacology of Antineoplastic and Other Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. R. Bachur	Head, Biochem. Sect.	LPH, NCI
OTHER:	S. D. Reich	Clinical Associate	LPH, NCI
	C. E. Riggs Jr.	Biologist	LPH, NCI
	P. H. Wiernik	Head, Med. Oncol.	CL, NCI
	P. Chang	Clinical Associate	CL, NCI
	H. Van Vunakis	Brandeis University	
	J. J. Langone	Brandeis University	

COOPERATING UNITS (if any)
Medical Oncology, Baltimore Cancer Research Center, NCI
Biochemistry Department, Brandeis University

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Biochemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

SUMMARY OF WORK (200 words or less - underline keywords)

Our sustained investigation of the clinical pharmacology of antineoplastic agents continues to aid in the investigation of fundamental findings to clinical problems. We have elucidated a major portion of the human biotransformation pathway of adriamycin. This involves carbonyl reduction, reductive glycosidic cleavage, hydrolytic glycosidic cleavage, O-demethylation, O-sulfation, and O-β-glucuronidation. We have aided in the application of a radioimmunoassay for the anthracyclines in clinical studies and have established new pharmacologic methohds and parameters for these agents.

PHS-6040
(12-75)

Project Description:
To study the disposition of antineoplastic and other drugs in humans with respect to absorption, distribution, biotransformation, and excretion. To characterize drug effects, potency, and maximum efficacy. To evaluate the toxicity of drugs to aspects of biological variations, and other factors such as age, body weight, sex, route of administration, time of administration, rate of inactivation and excretion, tolerance, pathological state, genetic factors, and drug-drug interaction. To develop techniques to minimize toxic effects of drugs without effecting the therapeutic value.

Human urinary metabolites which retain adriamycin's specific fluorescence properties were isolated and identified. Metabolites were extracted by adsorption to polysytrene polymeric sorbants, separated on silicic acid columns and purified by thin layer chromatography. Structures were determined by comparative chromatography; infrared, fluorescence, and mass spectroscopy; and enzymatic and chemical degradation. Substances identified were adriamycinol, adriamycinol aglycone, adriamycin aglycone, deoxyadriamycin aglycone, deoxyadriamycinol aglycone, demethyldeoxyadriamycinol aglycone, demethyldeoxyadriamycinol aglycone 4-O-sulfate, and demethyldeoxyadriamycinol aglycone 4-O-β-glucuronide. Other metabolites have been purified but not identified. Human metabolism of adriamycin involved carbonyl reduction, reductive glycosidic cleavage, hydrolytic glycosidic cleavage, O-demethylation, O-sulfation, and β-glucuronidation. Carbonyl reduction was the major enzymatic conversion occurring in the human.

High-pressure liquid chromatography (HPLC) is used to separate adriamycin and the metabolites, in the urine of these patients. The concentrations of the isolated compounds are determined by radioimmunoassay (RIA). Their excretion rates decrease with time and their relative molar ratios vary in collections made at different times and from different individuals. This combined HPLC-RIA system offers an efficient and sensitive procedure for separation and estimation of these compounds that is not subject to interference from nonrelated endogenous materials which may affect results obtained with other methods.

Patients with disseminated sarcomas were treated with adriamycin according to an intermittent single high-dosage schedule based on our prior pharmacologic observations. Adriamycin is an effective agent in the therapy of disseminated sarcomas and can be used with relative safety by following pharmacologic principles.

We will continue to study the clinical pharmacology of cancer chemotherapeutic agents and attempt to relate fundamental laboratory findings to clinical observations.

Publications:
Langone, J.J., Van Vunakis, H., and Bachur, N.R.: Adriamycin metabolites separation by high pressure liquid chromatography and quantitation by radioimmunoassay. Biochem. Med. 12: 283-289, 1975.
Reich, S.D. and Bachur, N.R.: Contact dermatitis associated with adriamycin and daunrubicin. Cancer Chemother. Rep. 59: 677-678, 1975.
Takanashi, S. and Bachur, N.R.: Adriamycin metabolism in man: Evidence from urinary metaboiltes. Drug Metab. Disp. 4: 79-87, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06250-03 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

TEM, SEM, and Culture of Human Leukemic Cells and of Normal Donor Granulocytes for Transfusion Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. T. Sanel	Research Biologist	LPH, NCI
OTHER:	P. H. Wiernik	Head, Med. Oncol.	CL, NCI
	J. Aisner	Clinical Associate	CL, NCI
	C. A. Schiffer	Clinical Associate	CL, NCI
	M. J. Egorin	Clinical Associate	LPH, NCI
	R. L. Felsted	Senior Staff Fellow	LPH, NCI
	N. R. Bachur	Head, Biochem. Sect.	LPH, NCI

COOPERATING UNITS (if any)
Medical Oncology, Baltimore Cancer Research Center, NCI

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Biochemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1/4	1/8	5/8

SUMMARY OF WORK (200 words or less - underline keywords)

In four cases submitted for our study, we demonstrated the accepted micromorphological criteria for Hairy Cell Leukemia, mononuclear clear cells with numerous cytoplasmic projections at the periphery. Zipper-like junctions were demonstrated in these cells as well as their ability to phagocytose microorganisms. These are properties of cells of the monocytic series, and strongly suggest the non-lymphoid origin of Hairy Cells. Adhesiveness to glass and plastic and responsiveness to isolectins were demonstrated in all cases. Glucose utilization and thymidine-uptake were determined.

Cells obtained by filtration leucapheresis using current methods were demonstrated by SEM, TEM, cyto-chemical and functional testing to be altered during harvesting and processing for transfusion. In a model system we demonstrated that washing harvested cells in HBSS and brief incubation in heparinized autologous plasma significantly restored them structurally and functionally.

Project Description:
Continuing studies are directed toward the characterization of leukemias of uncertain identity by ultrastructure, cytochemistry and function of peripheral blood and bone marrow cells. Hairy cell leukemia should be considered in suspected cases since the prognosis for untreated patients with this disease is considerably better than for those treated chemotherapeutically.

In four cases characterized by leukemic [illegible] resistant acid phosphatase submitted for our study, we [illegible] criteria for hairy cells in leukemic [illegible]. Properties of normal and leukemic monocytes were demonstrated in three cases as follows: [illegible] were demonstrated in one case, phagocytic [illegible] in two cases, and scanning electron microscopy of cells in [illegible] ability to adhere to plastic or glass were positive for most [illegible]. Migration to calcitin was demonstrated and glucose utilization and [illegible] were determined in all cases.

Functional, histochemical and morphological characteristics of [illegible] cells of a patient with acute monocytic leukemia was correlated with [illegible]. The presence of morphologically and functionally differentiated [illegible] tic cells may account for the marked tissue invasion in some patients with [illegible] cytic leukemia.

To evaluate the suitability for transfusion [illegible] harvested on nylon fibers and treated by various techniques in a model system to restore them to levels comparable to control cells in micromorphology, viability and function, and to, if possible, eliminate factors inducing adverse reactions in recipients. Cells obtained by filtration leucapheresis using current methods were [illegible] M, TEM, cytochemical and functional testing to be altered [illegible] harvesting and processing for transfusion. In a model system, we demonstrated that washing harvested cells in HBSS and brief incubation in heparinized autologous plasma [illegible] restored them structurally and functionally. Preliminary clinical trials of this method have been made and results are encouraging.

In order to determine the role of several components [illegible] granulocyte adhesion to nylon fibers conventionally used in [illegible] from two mouse strains genetically deficient in C 5 were [illegible] miniaturized model system. Granulocyte adhesion occurred but the significance of differences between controls and deficient species remain to be evaluated.

We will continue our investigation of methods to harvest [illegible] and restore the maximal viability and efficiency of granulocytes collected for [illegible] therapy.

Publications:
Sanel, F.T., Aisner, J., Schiffer, C.A., and Wiernik, P.H.: [illegible] of Granulocytes Harvested by Filtration Leucapheresis: Functional, Histochemical, and Ultrastructural Studies. In Goldman, J. (Ed): Leucocyte Separation and Transfusion. London, Academic Press, 1975, pp. 236-250.
Schiffer, C.A., Sanel, F.T., Stechmiller, B.K., and Wiernik, P.H.: Functional and morphologic characteristics of the leukemic cells of patients with acute monocytic leukemia: Correlation with clinical features. Blood 46: 49-70, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06272-05 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Studies of Myelomonocytic Leukemia in an Animal Model System: Light and Electron Microscopy, Biological Experiments and Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F. T. Sanel	Research Biologist	LPH, NCI
OTHER	P. H. Wiernik	Head, Med. Oncol.	CL, NCI

COOPERATING UNITS (if any)
Medical Oncology, Baltimore Cancer Research Center, NCI

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Biochemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1/2	1/8	3/8

SUMMARY OF WORK (200 words or less - underline keywords)

Transplacental passage of WEHI-3 tumor viruses induce lymphomas in offspring suggesting that immunologic responses to them have not been elicited; adult BALB/c mice cannot reject these tumor-inducing viruses; these viruses are in some instances sequestered and/or produced within the bone matrix; demyelinization of spinal cord axons and the presence of virus in the spinal cord may be related phenomena in animals that develop the hind leg paralysis.

Preliminary chemotherapeutic studies using Levamisole (Ortho). Latency periods of tumor development did not differ significantly from tumor injected controls.

PHS-6040
(12-75)

Project Description:
To study etiology, passaging under varying conditions and cross-studies grafting of transplantable, muramidase-producing myelomonocytic [illegible] virus producing cells utilizing techniques of short term tissue culture, hematological, histological, histochemical and ultrastructural techniques; species identification of the specific virus type within the murine oncorna virus group, and further evaluation of their oncogenic capacity; to determine the phagocytic competence of myelomonocytic neoplastic cells whose normal counterparts actively ingest particulates; to determine the effect of Levamisole (Ortho) on tumor induction and/or regression.

Scanning electron microscopy (SEM) of tissue cultured AKR-1 tumor cells additionally confirmed their identity as monocyte-macrophage type cells and transmission (TEM) demonstrated their production of "C" type particles, previously noted in tumor extracts that demonstrated group specific antigens of the C-type group and demonstrated high titers of infectivity. Newborn C3H/He mice injected with NA tropic cell-free tumor filtrates in many instances developed hind leg paralysis and lumbar spinal cord samples revealed moderate demyelinization and extracellular C-type viruses. TEM of decalcified femurs of osteopetrotic animals revealed numerous C-type particles in lacunar areas where osteocytes were embedded in bone matrix. Transplacental transmission of viruses by injection of pregnant females with cell free filtrates (CFF) resulted in lymphomas in both mothers and offspring after latencies of 6 to 18 months. Injection of CFF into normal 10 month old AKR/J male mice induced lymphomas after a 6 to 8 month latency. In preliminary chemotherapeutic studies with Levamisole (Ortho), the drug was administered [illegible] mouse at stages intervals before tumor grafting, simultaneously with tumor grafting, and after tumor grafting, and/or in multiple doses of [illegible]. Latency periods of tumor development did not significantly differ from tumor injected controls.

These studies have established that transplacental passage of C-type tumor viruses induce lymphomas in offspring suggesting that immunologic responses to them have not been elicited; that adult BALB/c mice [illegible] C-type tumor-inducing viruses, unlike adult AKR mice injected with Gross virus; that C-type viruses are in some instances sequestered and/or produced within the bone matrix; and that demyelination of spinal cord axons and the presence of virus in the spinal cord may be related phenomena in animals that develop the hind leg paralysis.

We will continue to study the oncogenic expression of these viruses and will investigate in vivo and in vitro chemotherapeutic anti-viral and anti-neoplastic agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06924-02 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Ultrastructural Studies of Aberrant Cells in Possible Preleukemic States

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: F. T. Sanel — Research Biologist — LPH, NCI

OTHER: J. Breton-Gorius
M. Y. Daniel
J. Chevalier
M. Lebeau
G. Flandrin

COOPERATING UNITS (if any)
Institut de Recherches Sur Les Maladies du Sang, Creteil and Paris, France

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Biochemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1/8	1/8	3/4

SUMMARY OF WORK (200 words or less - underline keywords)

The abnormal erythroid precursors from the bone marrow of a patient with refractory anemia and ineffective erythropoiesis were studied and characterized. Ultrastructural studies in our laboratories of material sent to us from France revealed unusual junctional complexes between aberrant mono- and mutlinucleated erythroid precursors and similar seemingly intracellular structures resembling the zipper-like junctions we reported (Science 168, 1970) in human leukemic monocytes.

PHS-6040
(12-75)

Project Description:
To study and characterize the abnormal erythroid precursors from the bone marrow of a patient with refractory anemia and ineffective erythropoiesis. Ultrastructural studies in our laboratories of material sent to us from France, revealed unusual junctional complexes between aberrant mono- and multinucleated erythroid precursors and similar seemingly intracellular structures resembling the zipper-like junctions we reported (Science 168, 1970) in human leukemic monocytes.

Continuing ultrastructural and cytochemical studies are in progress on peripheral blood and bone marrow cells from patients exhibiting possible hematologic dyscrasias in order to elicit, if possible, predictive factors toward definitive diagnosis that would aid in prognosis and treatment.

Publications:
Breton-Gorius, J., Flandrin, G., Daniel, M.T., Chevalier, J., Lebeau, M., and Sanel, F.T.: Septate-like junctions in abnormal erythrocytes. Virchow's Arch. B. Cell. Pathol. 18: 165-180, 1975.

SMITHSONIAN CLINICAL INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

Z01 CM 00210-06 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Development of RNases as an Aid in Sequencing of RNA from Leukemia Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C.C. Levy Chief LPH, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Pharmacology, BCRC

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the study was the isolation from microbial sources of nucleases which will hydrolyze long chain nucleic acids at specific base linkages as an aide in the sequential analysis of RNA from various murine leukemia viruses.

PHS-6040
(12-75)

Project Description:

The technique of enrichment culture was employed to isolate a number of microorganisms from soil which could degrade RNA at specific nucleotide bonds. Thus far, nucleases specific for bonds containing cytidylic acid and uridylic acid have been obtained from two of the organisms. A third, Serratia sp., elaborates an enzymes which shows preferential specificity for adenylic acid linkages. The second of the two organisms described, Citrobacter sp., which hydrolyzes uridylic acid internucleotide bonds, has shown interesting alterations in its specificity under the influence of the polyamine, spermidine. The Citrobacter nuclease shows no activity towards polycytidylic acid until spermidine is introduced into the reaction mixture. At this point, the degradation of cytidylic acid containing nucleotide bonds is rapid and complete. Of considerable interest is that this is the first instance in which spermidine has been shown to have a direct effect on an enzyme. Of all the polyamines tested, the enzyme binds spermidine preferentially. The binding of the polyamine to the enzyme is the first case known where spermidine is bound to a protein. This binding has enable us to isolate the peptide region of the enzyme to which the polyamine is bound. Two peptides have been isolated and the amino acid composition of both have been determined. It has been shown that for every mole of spermidine bound, one mole of peptide can be isolated.

Nucleic acid metabolism or, more correctly, the alteration of nucleic acid metabolism, is believed to play an important role in cancer induction. It is hope that an understanding of the sequential relationship of one nucleic acid to the other in the construction of a large chain polymer in tumor-causing viruses may provide a clue to the nature of cancer induction. In addition, the effect of spermidine on the enzyme which hydrolyzes polyuridylic acid is of considerable interest. The reason is that in the urine of patients with diagnosed cancer there appears to be an increase in polyamine levels. The relationship of spermidine to the Citrobacter enzyme may have significance in terms of the physiological changes which are occurring in the cell asthe tumor progresses. In addition, the ability of the protein to bind polyamines suggests that nucleases may play a considerably more pivotal role in the regulation of the cellular metabolism than had hitherto been suspected. This type of phenomena will be investigated in human enzymes as well, particularly in the enzymes that may be found in patients with cancer.

In addition, the finding that the RNases isolated, are inhibited by polyadenylic acid (poly A), is significant because this polynucleotide is at the 3' termini of mRNA. This would suggest that poly A can regulate the survival of mRNA in the cell by acting to modulate the activity of an RNase which could destroy it. Spermidine, moreover, will reverse this inhibition - lending credence to the view that this polyamine is impartial in cellular regulation of nucleic acid metabolism.

The projected course of this study is to utilize these enzymes as a means of sequencing RNA isolated from tumor viruses. It is hope that by establishing partial sequences in a number of RNAs from various tumor viruses, some relationship between the sequence and neoplasia will be seen.

Publications:

Schmukler, M., Jewett, P.B., and Levy, C.C.: The effect of polyamines on a residue-specific human plasma ribonuclease. J. Biol. Chem. 250: 2206-2212, 1975

Levy, C.C., Schmukler, M., Frank, J.J., Karpetsky, T.P., Jewett, P.B., Hieter, P.A., LeGendre, S.M., and Dorr, R.G.: Possible role for poly(A) as an inhibitor of endonuclease activity in eukaryotic cells. Nature 256: 340-342, 1975.

Levy, C.C., Roles of RNases in cellular regulatory mechanisms. Life Sciences 17: 311-316, 1975.

Karpetsky, T.P., Hieter, P.A. Levy, C.C.: Role for polynucleotides as inhibitors of endonuclease activity. In Proc. Second Internatl. Conf. on Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, in press, 1976.

Frank, J.J., Hawk, I.A., and Levy, C.C.: Peptides isolated from Enterobacter nuclease as potential polyamine binding sites. Biochim. Biophys. Acta, in press, 1976.

Hieter, P.A., LeGendre, S.M., and Levy, C.C.: The stabilization of an RNA molecule by 3'-terminal Poly(A)-induced inhibition of RNase activity. J. Biol. Chem., in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06242-06 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Polyamines and Cancer; Functional Significance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Karpetsky	Staff Fellow	LPH, NCI
OTHER:	C. C. Levy	Chief	LPH, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology, ECRC

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

SUMMARY OF WORK (200 words or less - underline keywords)

The diagnostic utility of putrescine, spermidine, cadaverine, and spermine as cancer biomarkers was investigated. Levels of these polyamines in the serum of patients displaying a variety of malignancies were measured using a new polyamine analyzer (Technicon). The presence, in serum, of unidentified polyamines with retention times close to those for cadaverine and spermidine makes interpretation of concentrations of these two polyamines equivocal. Levels of spermine are usually very low, while those of putrescine do not appear to reflect accurately the status of malignancy. Twenty-four hour urine collections were made from cancer patients and polyamines were determined in these samples. Preliminary data indicate that, using samples of urine, the resolution of peaks representative of the polyamines is considerably improved, relative to that obtained with serum samples. Studies are underway to determine if a reproducable relationship exists between the disease status of cancer patients and levels of individual polyamines from urine samples.

Project Description:

The concentrations of polyamines (putrescine, cadaverine, spermidine, spermine) in the serum and urine of cancer patients were determined. Serial or time-course studies of levels of these polyamines were done using a number of patients in order to establish whether or not these compounds are useful either in the diagnosis of cancer or in the determination of status of malignancy or efficacy of treatment. Analysis of levels of individual polyamines was undertaken using a Technicon polyamine analyzer. For the measurement of any different polyamine, sample handling techniques enable the measurement of either free (unbound) polyamine or total (free and bound) polyamine. Levels of free polyamine were found to be much lower than those of total polyamine, hence, for routine purposes in our study, the latter were utilized.

Levels of individual polyamines in serum were difficult to assess due to the fact that, in most of the samples, unidentified polyamines have retention times close to those for cadaverine and spermidine, thus obscuring these peaks. No relationship could be found between status of malignancy or course of chemotherapy and serum polyamine levels.

Urinary levels of polyamines are easier to evaluate because the individual compounds are well resolved in most cases. Initial studies indicated that the polyamines are elevated in patients with cancer. However, putrescine levels appear to reflect both chemotherapy and state of disease. Spermidine levels appear to accurately reflect the state of disease, being elevated above normal control values prior to treatment, then falling to near normal values as remission of disease is obtained. Spermine levels are always very low and these measurements are not useful indicators of state of disease or course of treatment. Measurements of overall polyamine levels are misleading since the levels of putrescine can change drastically when antibiotics are administered, thus altering the total or overall polyamine levels.

Time course studies of individual polyamine levels in the urine of cancer patients will be continued. Attention will focus on the derivation of a useful relationship between disease status and polyamine level. Comparison of polyamine levels with serum RNase levels will be made. This enzyme is a marker for renal impairment (Z01 CM 06933-02 LPH) and, as such, serves as an important parameter which may relate to polyamine levels.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06247-06 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Purification and Study of Nucleases with Differing Residue Specificities

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Schmukler	Senior Investigator	LPH, NCI
OTHER:	C.C. Levy	Chief	LPH, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pharmacology, BCRC

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

This project has three primary aims: first, and originally, to seek nucleases, which by virtue of their specificity, would be useful as tools in sequencing nucleic acids; second, to study the factors involved in regulation of nucleic acid metabolism; and third, to study alterations in nucleic acid metabolism in malignancy in order to determine whether any of these changes could serve as indicators for the presence or absence of disease.

Project Description:

Human plasma and pancreatic ribonucleases have been purified extensively and been compared. Many of their properties are similar. Molecular weights, substrate specificities, and effects of various substances such as synthetic polyribonucleotides and polyamines are being studied. Endogenous inhibitors of nuclease activity have been found in human tissues and investigations are in progress to characterize these substances and to determine their physiological significance in the control of nucleic acid metabolism. In clinical studies, it has been found that many individuals with or having a history of malignancy show elevated plasma nuclease levels. Studies are in progress to verify these early findings and to determine whether a reliable index of malignant activity can be found.

A human plasma ribonuclease purified more than 2000-fold is potently inhibited by synthetic polyribonucleotides such as poly G, poly X, poly A, poly (A,G), poly (G,I), and poly (G,U). Low concentrations of the polyamines, spermine, and spermidine, are able to reverse these inhibitions. These polyamines also cause a reduction in the molecular weight of the enzyme. An inhibitor of this enzyme has been found in human tissues, and its effect is also reversed by polyamines. Plasma ribonuclease activities are elevated in 70% of individuals with malignant disease.

Publications:

Levy, C.C., Schmukler, M., Frank, J.J., Karpetsky, T.P., Hieter, P.A., Jewett, P.B., LeGendre, S.M., and Dorr, R.: A Possible Role for Poly A as an Inhibitor of Endonuclease Activity in Eukaryotic Cells. Nature 256: 340-342, 1975.

Drake, W.P., Schmukler, M., Pendergrast, W.J., Jr., Davis, A.S., Lichtenfeld, J.L., and Mardiney, M.R., Jr.: Abnormal Profile of Human Nucleolytic Activity as a Test for Cancer. J. Natl. Ca. Inst. 55: 1055-1059, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06903-03

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Development of DNases as an Aid in the Sequencing of DNA from Viral Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Levy	Chief	LPH, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology, BCRC

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the study was the isolation from microbial sources of a DNase which will hydrolyze deoxyribonucleic acid at a specific base linkage as an aid in the sequential analysis of DNA obtained from a tumor virus.

Project Description:

The technique of enrichment culture was employed to isolate DNases from a microorganism which degrades DNA at specific nucleotide linkages. The organism has been classified as a Flavobacter and the enzyme has been characterized to some degree. The enzyme has a molecular weight of at least 200,000 and presumably breaks up into four, perhaps six, subunits. The enzyme appears to be an endonuclease in that alteration of the termini of various substrates has no effect on enzyme activity. The enzyme has strong specificity for deoxyadenylic acid, since it will not attack ribonucleic acid or polyribonucleotides under any conditions. The enzyme should be helpful in elucidating the primary sequence of DNA from tumor viruses. The enzyme, purified 2600-fold, will bind the polyamines, particularly spermidine. The binding site on which the polyamine adheres to the protein has been found to be nucleophilic in nature. Thus this finding indicates that not only RNases, but also DNases can bind polyamines. The binding site, at least with respect to the amino acid composition, appears to be generally similar. The significance of this finding is currently being explored.

The ability to degrade deoxyribonucleic acid at specific linkages when the nucleic acid is obtained from viral sources should be of intense interest in sequencing the DNA of a tumor virus. It is hoped to compare regions of the DNA with comparable areas isolated from RNA tumor viruses.

The isolation of these enzymes has, in addition, given some information on the nature of extrachromosomal reproduction in cells. That is, the enzyme appears to be in a plasmid in the organism. This structure consists of circular duplex DNA to which the DNase is bound. We are currently attempting to learn if the plasmid codes for the enzyme bound to it.

It is proposed to isolate a homogenous DNA from viral sources and to attempt a sequential analysis of the nucleotide composition. An attempt will be made to sequence the peptide isolated which binds spermidine and see the significance of this binding site with respect to the binding site on the ribonucleases. Since at the same site large ordered polynucleotides (poly AU) bind to the protein, it is of interest to determine if this binding site is the same and, if the same, is this a general phenomena throughout biological proteins? Would, for example, the binding site to which poly AU adheres be the same on a membrane surface, a surface which is of considerable importance in human chemistry?

Publications:

Karpetsky, T.P., Hieter, P.A., and Levy, C.C.: Role for polynucleotides as inhibitors of endonuclease activity. In Proc. Second Internatl. Conf. on Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06925-03 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the Metabolism Function and Storage of Human Blood Platelets

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Schmukler	Senior Investigator	LPH, NCI
OTHER:	P.D. Zieve	Chief,	Dept. of Medicine, Balto. City Hospitals

COOPERATING UNITS (if any)

Department of Medicine, Baltimore City Hospitals, Baltimore, Maryland 21224

LAB/BRANCH
Laboratory of Pharmacology, BCRC

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

SUMMARY OF WORK (200 words or less - underline keywords)

These studies are designed to achieve several objectives: first, to obtain metabolic, physiologic, and pharmacologic information which would aid in the preservation and transfusion of stored platelets; second, to study the relation of various biochemical and pharmacological factors on the function of platelets as measured by the release reaction; third, to elucidate the mechanism of the release reaction; and fourth, to gain general information about the mechanism of membrane-mediated cellular reactions.

PHS-6040
(12-75)

Project Description:

Studies of the effects of hydroxyethyl starch on the function of human platelets were extended to DMSO, another sustance considered for use in platelet storage. DMSO, at low levels, also does not adversely affect the platelet release reaction. The interactions of con A and thrombin and their effects on platelet function have been studied. Con A binding to platelet membrane surfaces has been shown to be necessary before con A can initiate the release reaction. Further studies of con A receptors on platelet membranes are contemplated. Studies have been initiated to develop a simple technique for the presence of platelet antibodies in order that incompatible transfusions may be avoided. Other studies are in progress to determine the effects of various plasma substances on platelet function.

Publications:

Schiffer, C.A., Aisner, J., Schmukler, M., Whitaker, C.L., and Wolff, J.H.: The effect of Hydroxyethyl Starch on In Vitro Platelet and Granulocyte Function. Transfusion 15: 473-476, 1975.

Schmukler, M., Zieve, P.D., and Jewett, P.B.: Induction of the Platelet Release Reaction by Conconovalin A: Relation of Con A Binding to Release and Modification of Release by ATP, PGE, and Amantadine. Biochem. Pharmacology, in press.

MATHEMATICAL GRANTS INFORMATION EXCHANGE PROJECT NUMBER (DO NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06926-03 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Role of Polyamines in Regulating Activity and Specificity of Various Nucleases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Karpetsky	Staff Fellow	LPH, NCI
OTHER:	C.C. Levy	Chief	LPH, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology, BCRC

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

SUMMARY OF WORK (200 words or less - underline keywords)

The polyamines regulate RNA levels by stimulating the hydrolytic activity of a number of bacterial and mammalian ribonucleases (RNases). These effects cannot be duplicated by metal ion. Polyamines can also control RNA levels by a mechanism involving RNase and purine inhibitors of this enzyme. The attachment of a 3' terminal polyadenylic acid (poly(A)) segment to an RNA molecule serves to inhibit the RNase mediated degradation of the non-polyadenylated portion of the molecule. Inhibition of RNase activity, and, therefore, substrate degradation, is dependent upon the length of the poly(A) segment. Spermidine reverses this poly(A) induced inhibition of RNase activity and thus, in the presence of this polyamine, there is an increase in the hydrolysis of the non-polyadenylated portion of the RNA. Spermidine can control, therefore, the rate at which a messenger RNA-like molecule is degraded by any of several RNases. Polyamines also serve to protect RNases from thermal inactivation and to renature inactivated enzyme.

Project Description:

Literature precedent indicates that the stability of messenger RNA (mRNA) is directly related to the existence of a 3'-terminal polyadenylic acid (poly(A)) sequence. Purine homopolymers such as poly(A) are known to inhibit the activity of a number of ribonucleases (RNases). The polyamine, spermidine, reverses this inhibition induced by poly(A). In order to test the hypothesis that the stability conferred upon mRNA by the poly(A) segment may be, in reality, protection from hydrolysis by RNase, a model system was constructed utilizing an RNase and an RNA having poly(A) covalently linked to the 3'-terminus. Six species of E. coli [^{3}H] 5S rRNA containing the 3'-terminal tracts of poly(A) ranging in size from 12 to 92 nucleotides in length were synthesized with primer-dependent polynucleotide phosphorylase. When these different [^{3}H] 5S rRNA poly(A) preparations were utilized as substrates for ribonucleases from either Enterobacter sp., bovine pancreas, or human spleen, it was found that the coupled poly(A) portion protects the remainder of the model mRNA molecule from hydrolysis. Increased inhibition of RNase with concomitant greater protection of substrate is found as the poly(A) segment is lengthened. However, since poly(A) is a very effective inhibitor, sequences containing as few as 12 adenylic acid residues elicit greater than a 60% inhibition of activity when either enzyme is utilized. The protection afforded the polynucleotide by even the largest terminal poly(A) tract is reversed in the presence of low concentrations (5×10^{-4} M) of spermidine, thus resulting in a rapid hydrolysis of the non-polyadenylated portion of the substrate. The increase in substrate hydrolysis induced by spermidine is a function of the length of the covalently bound poly(A) segment, with longer poly(A) tracts requiring higher concentrations of spermidine to overcome the inhibition of RNase activity. Thus, with respect to degradation by RNase, the stability of the non-polyadenylated portion of the substrate is highly dependent upon two factors: the length of the poly(A) segment which inhibits the activity of the RNase, and the concentration of spermidine which mediates the reversal of this inhibition. This model, utilizing poly(A) covalently bound to RNA, would be expected to be operational in any case in which the activity of an RNase is inhibited by polyadenylic acid and a polyamine causes reversal of this inhibition.

Publications:

1. Levy, C.C., Schmukler, M., Frank, J.J., Karpetsky, T.P., Jewett, P.B., Hieter, P.A., LeGendre, S.M., and Dorr, R.G. Nature 256: 340-341, 1975.

(INFORMATION EXCHANGE — DO NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06927-03 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Properties of the Enterobacter Nuclease: Interaction of Nucleases and Polyamines.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Frank	Staff Fellow	LPH, NCI
OTHER:	C.C. Levy	Chief	LPH, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

SUMMARY (200 words or less - underline keywords)

The purpose was to isolate a nuclease which can hydrolyze phosphodiester bonds at particular residues in RNA and would therefore be useful in determining the primary sequence of various types of ribonucleic acid. Also, it is of interest to examine the interaction of the nucleases with the polyamines since these agents have been found in elevated levels in the serum and urine of cancer patients. Spermidine previously had been shown to bind to and effect the activity of the Citrobacter nuclease. The effects of the polyamines on polynucleotide inhibition of ribonuclease activity was also explored. Two enzymes were of primary importance in these studies: the Enterobacter nuclease and the Staphylococcal nuclease.

Project Description:

The purpose was to isolate a nuclease which can hydrolyze phosphodiester bonds at particular residues in RNA and would therefore be useful in determining the primary sequence of various types of ribonucleic acid. Further, it was of interest to determine the interaction of nucleases with the polyamines which have been shown to bind to and affect the activity of Citrobacter nuclease.

The Citrobacter ribonuclease was isolated from Enterobacter sp. which had been obtained from soil using enrichment culture techniques.

Studies with the polyamines have shown dramatic increases in enzyme activity for assays run at low ionic strength while only modest enhancement of activity was observed at high ionic strength. The specificity of the nuclease was unaffected by the polyamines with either synthetic nucleotides or RNA as substrate. Inhibition of the enzyme by the ordered polynucleotides was reversed using spermidine or spermine, while other cations tested had no effect. The C ribonuclease was shown to bind to the polyamines and to polyguanylic acid. Tryptic digestion of the poly G-bound enzyme gave peptides bound to poly G which were released using excess spermidine. Threee spermidine-bound peptides were isolated after ion exchange chromatography and the nucleophilic amino acids, serine, glutamic acid and aspartic acid were shown to be present in each peptide after amino acid analysis.

The polyamines were also shown to activate Staphylococcal nuclease for degradation of both RNA and DNA in the absence of calcium ion. If low concentrations of calcium ion are added to the polyamine-activated enzyme, up to double the expected activity (based on additive values) is observed. The polyamines also reversed inhibition of Staphylococcal nuclease by the ordered polynucleotides.

It is known that the polyamines are present in high concentrations in the urine of cancer patients. It is of great interest to determine how the polyamines affect the nucleases and their role in controlling the concentration of RNA in the cell. This information would be useful in controlling the growth rate of rapidly proliferating cells.The projected course is to determine more fully the nature of the binding of spermidine to the isolated peptides. Also, it is of interest to determine the location in the cells of the ribonuclease.

Publications:

Frank, J.J., Hawk, I.A., and Levy, C.C.: Polyamine Activation of Staphylococcal nuclease. Biochim Biophys. Acta 390: 117-124, 1975.

Frank, J.J., Hawk, I.A., and Levy, C.C.: Peptides Isolated from Enterobacter Nuclease as Potential Polyamine Binding Sites. Biochim. Biophys. Acta (in press) 1976.

Neuwelt, E.A., Frank, J.J., and Levy, C.C.: The purification of human spleen ribonuclease by immunoabsorption: identity of the enzyme with human liver ribonuclease. J. Biol. Chem., in press, 1976.

Frank, J.J. and Levy, C.C.: The properties of human liver ribonuclease: Polyadenylic acid-induced inhibition of the enzyme. J. Biol. Chem., in press, 1976.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 CM 06933-02 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Influence of Renal Insufficiency on Levels of Human Serum Ribonuclease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.P. Karpetsky	Staff Fellow	LPH, NCI
OTHER:	C.C. Levy	Chief	LPH, NCI
	R.L. Humphrey	Staff	Johns Hopkins University Medical School

COOPERATING UNITS (if any)

Johns Hopkins Hospital, Oncology Department

LAB/BRANCH
Laboratory of Pharmacology, BCRC

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

SUMMARY OF WORK (200 words or less - underline keywords)

The activity of serum ribonuclease (RNase) was measured in 34 patients with far advanced multiple myeloma and differing degrees of renal insufficiency. RNase levels are not a biomarker for the extent of plasma cell tumor. However, a strong inverse correlation was observed between the degree of renal impairment (expressed as creatinine clearance) and serum RNase levels. Time course studies of individual multiple myeloma patients indicated, without exception, that RNase levels reflect the status of renal function and not the extent of myeloma. Levels of this enzyme, therefore, are not of use in determining the response of tumor to treatment. At present, determinations of RNase levels in patients with leukemia or other malignancies are in progress with the intent of proving that RNase levels are not a biomarker for any of these types of cancer, and also that increases above normal values of levels of this enzyme are an early indicator of impending renal distress.

Project Description:

The purpose of this study was to examine the utility of serum RNase levels as a biomarker for the presence and status of cancer. Accordingly, RNase activity was measured in the serum of 34 patients, all of whom had far advanced multiple myeloma and, on whom complete medical histories were available. All had bone marrow plasmacytosis > 50% of nucleated cells and all studies were done at least four weeks after previous chemotherapy. RNase levels ranged from 12 to 456 units/ml/min (1 u = ΔA_{260} increment of 1.0) and the enzyme was specific for poly (C). All of 15 patients with creatinine clearance (C_{CR}) < 50 ml/min had an elevated RNase level (> 45 u/ml/min) relative to values determined in normal volunteers. Twelve of 19 patients with C_{CR} > 50 ml/min had normal RNase concentrations. The RNase ranged from 50-117 u/ml/min in the remaining 7 patients with C_{CR} > 50. A strong inverse correlation was therefore observed between the degree of renal insufficiency (C_{CR} values) and serum RNase levels. Several myeloma patients were studied throughout the course of their illness and the rise and fall of RNase was clearly related to the status of their renal function. Normal controls and patients with severe renal insufficiency but without plasma cell tumors were also studied. The serum of patients with bilateral nephrectomies had the highest levels of enzyme measured, indicating that some other tissue than the kidney is the origin of serum RNase.

The overall finding of this study is that RNase levels are not a biomarker for the extent of plasma cell tumor, but are an indicator of renal function. These results are confirmed on examination of serum RNase levels in patients with forms of cancer (AML, ALL, aplastic anemia, oat cell carcinoma) other than multiple myeloma. Evaluation of the results of in depth time course studies of serum RNase levels in individual cancer patients indicates that levels of this enzyme may be an early indicator of impending renal damage and may precede creatinine levels or creatinine clearance values, in this respect, by 1-3 days. Further studies are underway to confirm this finding.

Publications:

Karpetsky, T.P., Humphrey, R.L., and Levy, C.C.: Influence of renal insufficiency on levels of serum ribonuclease (RNase) in patients with multiple myeloma. Amer. J. Med., in press, 1976.

Karpetsky, T.P., Hieter, P.A., and Levy, C.C.: Role for polynucleotides as inhibitors of endonuclease activity. In Proceedings of Second Conference on Modulation of Host Resistance in the Prevention or treatment of Induced Neoplasms (Edit. by Chirigos, M.A.), in press, 1976.

Karpetsky, T.P., Hieter, P.A., Frank, J., and Levy, C.C.: Control of RNA levels by polyamines. Nature, in press, 1976.

Karpetsky, T.P., and Levy, C.C.: Control of RNAse with time: regulation by spermidine. Biochem. Biophys. Acta, in press, 1976.

Karpetsky, T.P., and Levy, C.C.: Thermal inactivation of a Citrobacter ribonuclease: temporal control of enzyme activity. Arch Biochem. Biophys., in press, 1976.

	NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06934-02 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

An Endogenous DNA Inhibitor of Citrobacter sp. Ribonuclease

NAMES, LABORATORY AND INSTITUTIONAL AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Levy	Chief	LPH, NCI
OTHER:	T.P. Karpetsky	Staff Fellow	LPH, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	0.6	1.5

SUMMARY OF WORK (200 words or less - underline keywords)

A ribonuclease (RNase) bound to an endogenous DNA inhibitor has been isolated from Citrobacter sp. The formation of the enzyme-inhibitor (EI) complex is not an artifact of the isolation procedure. Separation of the enzyme and DNA inhibitor was accomplished by phenol extraction of the EI complex. The enzyme has been previously purified to homogeneity. Characterization of the isolated inhibitor revealed that it is an heterogenous array of DNA molecules of small (9-11S) average size. As the temperature is raised, there is a large increase in the absorbance of the DNA inhibitor, indicating the double-stranded nature of this extrachromasomal DNA. Studies performed on the EI complex demonstrate that the DNA, which resembles a mini-plasmid, inhibits RNase activity by either a competitive (using Tris-HCl buffer) or a mixed (using potassium phosphate buffer) mechanism. K_i is a strong positive function of ionic strength with the consequence that increases in buffer concentrations reverse the inhibition of RNase activity with concomitant increased hydrolysis of substrate. Spermidine will also reverse inhibition, but at much lower concentrations than expected for an effect mediated by ionic strength alone.

Project Description:

Previous studies demonstrated the presence of an inhibitor bound to a ribonuclease (RNase) isolated from Citrobacter sp. The purpose of this current effort was to isolate and characterize this inhibitor. Two methods were utilized for the preparation of the enzyme-inhibitor (EI) complex. On one hand, the cells were broken using a French press and the supernatant after centrifugation was treated with protamine sulfate, then ammonium sulfate and the EI complex was isolated after gel filtration. Another method of preparing the EI complex involves treating the cells with lysozyme in a medium containing a non-ionic detergent. This procedure is often utilized to prepared plasmids and will not cause the release of chromasomal DNA. The isolation of the EI complex under these gentle conditions indicates that the formation of this complex is not an artifact of the isolation procedure. The EI complex could be dissociated by phenol extraction. The inhibitor was then treated with RNase A and subjected to gel filtration a final time. The inhibitor is not attacked by several RNases or by proteolytic enzymes. However, this DNA inhibitor is rapidly hydrolyzed by either DNase I or II. The double-stranded nature of the inhibitor is revealed by its melting profile (Tm = 90°, hyperchromicity = 30%, 10^{-2} M Tris-HCl, pH 7.5). The EI complex sedimented at 16S on sucrose density gradient centrifugation. The purified inhibitor sedimented as a single peak at 9-11S, indicating the small size of the plasmid-like DNA.

The effect of the inhibitor on RNase activity is strongly influenced by ionic strength. Increasing concentrations of Tris-HCl buffer (pH 7.1) reverse inhibition by increasing K_i alone. Although potassium phosphate buffer (pH 7.1) will also reverse inhibition, both K_i and Vm are affected by gradients of this salt. Kinetic mechanisms were established for the effects of both buffers. Spermidine will reverse inhibition of the RNase, but at much lower concentrations than required for an effect based solely on ionic strength. The plots of v vs spermidine are sigmoidal and the reciprocal plots are non-linear. This kinetic evidence suggests a mechanism in which more than one spermidine binds to the enzyme-inhibitor complex, giving rise to a number of enzyme-spermidine forms that can catalyze the substrate-product conversion. The association of the enzyme with inhibitor lengthens the half-life of denaturation at 37° and therefore protects the enzyme from thermal inactivation. This endogenous inhibitor is therefore of prime importance in regulating the amount of enzyme activity available at a given time.

At the present time, further studies are concentrating on the relationship between the DNA inhibitor and the RNase. In order to determine how widespread is the phenomenon of association of an enzyme with an extrachromasomal DNA inhibitor, an inducible DNase is being sought from bacteria. Whether or not an EI-complex consisting of DNase and DNA inhibitor can be isolated from this system will indicate if inducible enzymes bind to small DNA molecules.

Publications:

Hieter, P.A., Karpetsky, T.P., and Levy, C.C.: An endogenous DNA inhibitor of Citrobacter sp. ribonuclease. J. Biol. Chem., in press, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06935-02 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1975

TITLE OF PROJECT (80 characters or less)

Isolation and Characterization of Human Liver and Spleen Ribonucleases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Frank	Staff Fellow	LPH, NCI
OTHERS:	C.C. Levy	Chief	LPH, NCI
	E.A. Neuwelt	Clinical Associate	LPH, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pharmacology, BCRC

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.0	0.6

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the study was to isolate and characterize human ribonucleases in order to more fully understand their physiological role. Since ribonucleases have been found in elevated levels in serum and urine of cancer patients, characterization of the enzymes as to tissue of origin would aid in determining the interrelationship of ribonuclease activity and the disease state. A human liver enzyme was purified 2500-fold using poly G affinity chromatography. The spleen enzyme which was found to be antigenetically similar to the liver enzyme was purified on an immunoabsorbant containing bound antisera to liver enzyme. The enzymes were characterized and found to be very similar in their specificities and response to polynucleotide inhibitors and polyamines.

PHS-6040
(12-75)

Project Description:

The purpose was to isolate and characterize human liver and spleen ribonucleases to use for an investigation of factors contributing to control of RNA levels in the cell.

A ribonuclease has been purified 2500-fold from the 100,000 g supernatant of homogenized human liver cells. Standard purification procedures were used with a poly G affinity column as the final step. The pH maxima in phosphate buffer is a pH 6.4. Extensive hydrolysis of poly U produces 2', 3' cyclic phosphates. Prolonged digestion of C RNA indicated that 80% of the products consisted of oligonucleotides of less than 10 nucleotides in length. The 5' termini of the oligonucleotides were found to be the nucleosides, adenosine and guanosine, in a ratio of 4:5.

Studies with the polyamines have indicated that enzyme activity can be increased dramatically in assays run at low ionic strength. The polyamines can also reverse inhibition of enzyme activity by the polynucleotides at low ionic strength.

The human spleen enzyme was purified (700-fold) using antibody to human liver nuclease and found to be very similar in the enzymatic properties to the liver nuclease.

Since ribonuclease activity is a major factor in the determination of RNA levels in the cell, it is of interest to determine those factors which can affect RNase activity. With this information control of the turnover of RNA in rapidly proliferating cells might be possible. It was found that the poly A segments of synthetic and naturally occurring mRNA could inhibit enzyme activity in both cases.

Future studies will involve the use of the enzymes as markers of disease states in the two organs.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06936-02 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (50 characters or less)
The Properties of Protein Inhibitors of Ribonuclease Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. J. Frank	Staff Fellow	LPH-NCI
Other:	C. C. Levy	Chief	LPH-NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH:
Laboratory of Pharmacology, BCRC

SECTION:
Enzymology and Drug Metabolism Section, LPH, BCRC

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

SUMMARY OF WORK (200 words or less - Underline keywords)

The project is designed to examine protein inhibitors of ribonuclease activity in various human tissues. In order to better understand any role they might play in controlling ribonuclease levels in the serum and urine of cancer patients. RNase A affinity chromatography has proven to be the most effective means of isolating the inhibitors from tissue homogenates of liver brain and heart. The protein inhibitors will be characterized and compared to polynucleotide inhibitors for effectiveness in reducing hydrolytic activity of ribonucleases

PHS-6040

Project Description:

The purpose of the project is to investigate the mechanism of protein inhibition of ribonuclease activity, the stability of protein inhibitors and the control of inhibitor activity.

Protein inhibitors of nuclease activity have been isolated from rat liver and bovine lens. Kinetic studies indicate that the inhibition is of a non-competitive nature. The inhibitors were found to be unstable to freezing and thawing, but could be stabilized with albumin. Preliminary studies with putrescine, spermidine and spermine indicate that protein inhibition of nuclease activity is enhanced by these agents.

Protein inhibitors have been isolated from human brain, heart and liver (obtained post mortem) through the use of an RNase A affinity column. The brain and heart consistently showed an excess of inhibitor over ribonuclease, whereas, the amount of liver inhibitor varied dramatically with each specimen.

The proposed course is to characterize the various protein inhibitors and study their interrelationships. The relative inhibition by protein and polynucleotide inhibitors will also be examined.

Publications: None.

[illegible] INFORMATION EXCHANGE (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06945-01 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1975

TITLE OF PROJECT (80 characters or less)

Immunological studies of Human RNases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.A. Neuwelt	Clinical Associate	LPH, NCI
OTHER:	C.C. Levy	Chief	LPH, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology, BCRC

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

SUMMARY OF WORK (200 words or less - underline keywords)

Antisera to human RNases were produced. These antisera were used to immunologically characterize human RNases. They were also used to study the effects of poly(A) on the antigenicity of human RNases.

PHS-6040
(12-75)

Project Description:

In order to characterize human RNases, antibodies were made to the RNases purified from human liver, spleen, plasma, and pancreas. The antisera produced were used in several immunoassays, the results of which indicated that there are two antigenically distinct groups of RNases. The human spleen and liver enzymes were found to be immunologically identical and did not cross-react with human plasma and pancreas RNase. The human plasma and pancreas RNases were found to be immunologically similar, but not identical.

Additional studies were performed to study the interaction of RNases with antibody in the presence and absence of poly(A). These studies correlated very well with previous enzymatic studies of the effects of poly(A). The polynucleotide was able to inhibit RNase binding to antibody just as it had previously been shown to inhibit RNase activity. Similarly, the inhibition of RNase binding to antibody was reversed by spermidine just as spermidine had reverse inhibition of enzymatic activity in earlier studies. Therefore, poly(A) has a marked effect both on the catalytic and antigenic sitesof human RNases. Such effects are consistent with the hypothesis that the poly(A) present on the 3' terminus of mRNA has a regulatory effect on RNase activity.

Publications:

Neuwelt, E.A., Niziak, M.S., and Levy, C.C.: Immunological characterization of several human ribonucleases. Nature, in press, 1976.

Neuwelt, E.A., Frank, J.J., and Levy, C.C.: The purification of human spleen ribonuclease by immunoabsorption: identity of the enzyme with human liver ribonuclease. J. Biol. Chem., in press, 1976.

Neuwelt, E.A., Gahl, W.A., and Levy, C.C.: Immunological studies on the interaction of polyadenylic acid and human liver ribonuclease. Immunochemistry, in press, 1976.

PROJECT NUMBER (DO NOT use this space)

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06946-01 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Massive Infusion of Normal Human Lymphocytes into the Rabbit Subarachnoid Space

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.A. Neuwelt	Clinical Associate	LPH, NCI
OTHER:	C.C. Levy	Chief	LPH, NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Enzymology and Drug Metabolism

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

SUMMARY OF WORK (200 words or less - underline keywords)

Xenogenic and syngenic lymphocytes were infused into the subarachnoid space of rabbits. The toxicity and distribution of these cells were studied.

PHS-6040
(12-75)

Project Description:

Autologous lymphocytes have been shown recently to be specific and highly cytotoxic for human glioma. Yet pathological studies reveal that lymphocytes rarely penetrate glioma beyond the perivascular space, possibly because of the blood brain barrier. The present study was designed to examined the feasibility of circumventing this barrier by injecting massive numbers of lymphocytes directly into the subarachnoid space. Three to six billion lymphocytes were obtained from a normal human volunteer using the Haemonetics, Model 30, cell separator. After purification on ficoll Hypaque gradients, 100 million to one billion lymphocytes were injected cisternally into the subarachnoid space of a rabbit. Analysis of the CSF 36 hours after injection revealed a normal CSF sugar and 500 to 25,000 WBC/mm^3 of which 95+% were viable lymphocytes. CSF protein rose to as much as 500 mg%, but returned to less than 80-100 mg% by 2 weeks post-injection. A mild transient fever of 1-2^{o} C frequently developed and one of fifteen rabbits developed torticolli transiently; otherwise, the rabbits suffered remarkably little morbidity. It is conceivable, therefore, that the intrathecal administration of autologous sensitized lymphocytes following surgical decompression and exposure of a glioma tumor bed to the subarachnoid space may, with only minimal toxicity, provide lymphocytes with better access to tumor.

Publications: None.

[illegible] (Do NOT [illegible])	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06020-09 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Mechanism of Human Lymphocyte Reactivity to Non-Self

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Mardiney, Jr.	Head, Sect. Immunology & Cell Biology	LPH NCI
Other:	R.A. Winchurch	Staff Fellow	LPH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

SUMMARY OF WORK (200 words or less - underline keywords)

The continuation of work in this area has revolved about the analysis of lymphocyte responsiveness in vitro to a putative suppressive immunogenic virus (rubeola) and the meaning of this putative suppression in relation to observations within the clinical disease in man. In addition, work has been performed utilizing laser cytometry as a method of quickly and accurately evaluating cell change in vitro as a measure of cell responsiveness to specific antigen, mitogen or allogeneic cells.

Project Description:

In vitro experiments utilizing rubeola (1) demonstrated that this virus was not suppressive to human lymphocytes in vitro when exposed to specific antigen as previously reported. In addition, analysis of clinical data suggested reconsideration of the suppossed importance of transient dimunition of skin test reactivity during measles infection or immunization. The data is of importance since the concept has generally been held and reported that this virus routinely caused immunosuppression. The defects and conclusions relating to previous work by others was analyzed. The work is of importance since it delineates the proper sequence for evaluating such a phenomenon in the future. The need for uncontaminated antigen was also underlined (2). The application of laser cytometry to the evaluation of lymphocyte responsiveness in vitro was analyzed and compared to the standard thymidine assay. It was found that false conclusions relating to lymphocyte proliferation in vitro in response to specific antigen may occur when the standard thymidine assay is used alone. At times an inverse relationship existed between actual cell proliferation and thymidine incorporation. This was a surprising finding since the disparity was great and because opposite conclusions would have been drawn concerning the nature of immune responsiveness had the assay depended solely upon the measurement of thymidine incorporation alone.

Publications:

Dunmire, C., Ruckdeschel, J.C., and Mardiney, M.R., Jr.: Suppression of in Vitro Lymphocyte Responsiveness to Purified Protein Derivative by Measles Virus. Cell. Immunol. 20: 205-217, 1975.

Ruckdeschel, J.C., Kramarsky, B., and Mardiney, M.R., Jr.: Mycoplasma Contamination of Membrane Associated Measles Antigen. Inability to Demonstrate in Vitro Lymphocyte Resp nsiveness to Measles. Cell. Immunol. 20: 110-116, 1975.

[illegible] INFORMATION EXCHANGE (Do NOT use this space)	U.S. DEPARTMENT OF [illegible] NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06249 03 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Search for Antigen-Antibody Complexes in Renal Glomeruli of Tumor Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. C. Sutherland	Assistant Professor, Pathology	LPH NCI
Other:	M.R. Mardiney, Jr.	Head, Sect. Immunol. & Cell Biol.	LPH NCI

COOPERATING UNITS (if any)

Department of Pathology, University of Maryland Hospital
Baltimore, Maryland

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.25	.25

SUMMARY OF WORK (200 words or less - underline keywords)

The concept that subclinical immune complex disease may be present in the neoplastic state has been well defined by this laboratory. Within this context, antigen-antibody complex deposition occurs within glomerular basement membrane. Analysis of such deposits allows for determination of the specific antigen (s) involved. Current studies relate to the incidence of immune complex deposition in the kidneys of patients with Burkitt's lymphoma and the putative role of Epstein Barr Virus as antigen in such deposits.

PHS-6040
(12-75)

Project Description:

A total of 12 kidneys from Kampala, 10 from patients with Burkitt's lymphoma, 1 from a patient with Hodgkin's disease, and one from a patient with reticulum cell sarcoma were studied. Judgement relating to the presence or absence of immune complex deposition could be made in all but 1 kidney. Two of the Burkitt lymphoma kidneys had IgG and C'3 deposition in the glomeruli, 5 had IgG only and 1 had C'3 only. One Burkitt lymphoma kidney was negative for both IgG and C'3. The patient with Hodgkin's disease had IgG and C'3 deposition while the patient with reticulum cell sarcoma did not have IgG or C'3 present. All positivities were predominantly capillary in distribution and granular in pattern. As a group, the finding of complexes in 2 out of 9 (22%) of the Burkitt patients is analogous to that seen in patients who succumb to acute leukemia (18-20%). All kidneys (positive or negative) were stained with Epstein Barr Virus-associated antiserum. One Burkitt lymphoma kidney stained positively with this antiserum. This positivity could be abolished by a prior staining with unlabeled Epstein Barr Virus associated antibody but not with a non-specific antiserum.

Publications: None

PROJECT NUMBER (Do NOT use this ...)

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06274-05 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Characteristics of Reactivity of Tumor Specific Antibody in Vivo and In Vitro

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Mardiney, Jr.	Head, Sect. Immunology & Cell Biology	LPH NCI
Other:	W.P. Drake	Biochemist	LPH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	.5	.5

SUMMARY OF WORK (200 words or less - underline keywords)

Previous work has demonstrated the ability of complement to mediate the cross-reactive binding of tumor specific antibody to normal determinants. This in part has explained the lack of effectiveness of specific antibody to tumor in vivo - the specific antibody molecule binding to a non-tumor target (this binding mediated by complement). Further work has demonstrated that this phenomenon relates not only to tumor specific antigen but also relates to murine transplantation antigens.

PHS-6040
(12-75)

Project Description:

The latent homology of murine lymphoid antigens was revealed through complement mediated absorption of xenogeneic antiserum. Within this serum, rabbit xenoantiserum against C57BL/6 mouse spleen cells was initially absorbed with DBA/2 spleen cells in order to remove all activity against this target. Such antiserum had continued reactivity with C57BL/6 cells. When such antiserum, however, was further absorbed with DBA/2 cells in the presence of complement, activity against C57BL/6 cells diminished. This finding is of importance because it demonstrates a latent homology between determinants both on the C57BL/6 cell and DBA/2 cells over and above what had been considered previously. Such data can be explained on the basis of a weekly avid bond, highly reversible in the absence of complement, which becomes more avid in the presence of complement.

Publications:

Drake, W.P., Pokorney, D.R., Kopyta, L., and Mardiney, M.R., Jr.: In Vivo Decomplementation of Guinea Pigs with Cobra Venom Factor and Anti-C3 Serum: Analysis of the Requirements of C3 and C5 For the Mediation of Endotoxin-Induced Death. June, 1976, in press.

[illegible] EXCHANGE PROJECT NUMBER (DO NOT USE THIS [illegible])	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06292-03 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immune Adjuvants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Mardiney, Jr.	Head, Sect. Immunology and Cell Biology	LPH NCI
Other:	W.P. Drake	Biochemist	LPH NCI
	W.J. Pendergrast, Jr.	Senior Staff Associate	LPH NCI
	R.A. Winchurch	Staff Fellow	LPH NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	.75	.75

SUMMARY OF WORK (200 words or less - underline keywords)

In vivo and in vitro work was carried out to determine the further application and efficacy of synthetic polynucleotides on altering survival of high tumor strain mice as well as defining some of the in vitro effects of these agents upon the tumor cell.

Project Description:

Previous work had demonstrated that poly A:U was capable of markedly prolonging the survival of AKR mice and noted that this survival was age-dependent - the best therapeutic results occurring when this agent was initiated at 2 months of age. No beneficial effect was seen when it was initiated at 8 months of age. When female C3H/HeJ mice received weekly subcutaneous injections of 2 mg. polyadenylic-polyuridylic acid starting at 2 months of age, a result opposite to that obtained in the AKR model was seen. C3H/HeJ mice receiving subcutaneous injections of 2 mg. of polyadenylic-polyuridylic acid developed the spontaneous mammary carcinoma associated with this strain more rapidly. This data indicated that polyadenylic-polyuridylic acid has no generalized antineoplastic effect upon spontaneous tumors genetically associated with the specific murine strain. Additional work utilizing this agent within the EL4 model (C57BL/6) revealed that it inhibited tumor proliferation in vivo and directly inhibited thymidine incorporation of EL4 cells in vitro.

Publications:

Drake, W.P., Pendergrast, W.J., Jr., Kramer, R.E., and Mardiney, M.R., Jr.: The Age-Dependent Efficacy of Polyadenylic·Polyuridylic Acid Therapy Upon the Development of Spontaneous Leukemia in AKR Mice. Cancer Res. 36: 1172-1175, 1976.

Pendergrast, W.J., Jr., Drake, W.P., and Mardiney, M.R., Jr.: The Dependence of Successful Immunotherapy on Adequate Tumor Burden. J. Natl. Cancer Inst. 55: 1223-1225, 1975.

Drake, W.P., Pendergrast, W.J., Jr., Kramer, R.E., and Mardiney, M.R., Jr.: Enhancement of Spontaneous C3H/HeJ Mammary Tumorigenesis by Long-Term Poly A:U Therapy. Cancer Res. 35: 3051-3053, 1975.

Winchurch, R.A., Gibson, R., Godla, G., and Mardiney, M.R., Jr.: The Effect of Synthetic Polyribonucleotides on EL-4 Lymphoma. J. Reticuloendothelial Soc. 18:244-251, 1975.

	DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06932 01 LPH

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Correlation of Abnormal Nuclease Activity to the Prediction and Presence of Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.R. Mardiney, Jr.	Head, Sect. Immunology & Cell Biology	LPH NCI
Other:	W.P. Drake	Biochemist	LPH NCI
	D. Maor	Visiting Associate	LPH NCI
	W.J. Pendergrast, Jr.	Sr. Staff Associate	LPH NCI

COOPERATING UNITS (if any)

Tumor Immunology Branch, Surgery Branch, NCI, NIH, Bethesda, Maryland 20014

LAB/BRANCH
Laboratory of Pharmacology

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NCI, NIH, Baltimore, Maryland 21211

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

SUMMARY OF WORK (200 words or less - underline keywords)

The initial finding in this laboratory that an abnormal profile of nucleolytic activity is found in 5 strains of mice with a genetic propensity to develop tumor long before tumor is actually present; the elevation of RNase activity in transplantable tumor models; and the finding of a change in nucleolytic activity in the peripheral white cells of guinea pigs suffering from L2C leukemia caused us to look actively at the application of these findings as a possible diagnostic test for cancer. Further evidence for this association was recently demonstrated in a clinical trial in which an abnormal profile of serum nucleolytic activity was observed in a variety of patients with neoplastic disease.

PHS-6040
(12-75)

Project Description:

In order to determine whether an abnormal profile of nucleolytic activity would occur in a viral induced leukemia situation, Balb/c mice were infected with Friend Disease Virus and were studied subsequent to infection for the ability of lymphocyte cell-free lysates in serum to degrade polyuridylic and polycytidylic acid to acid soluble mono and oligonucleotides. Lymphocyte-associated RNase activity directed against both substrates was elevated by day 8 while serum nucleolytic activity was noticed to be largely unaffected by the progression of neoplastic disease. In all experiments with this model, this change in lymphocyte activity was the first observable reflection of neoplastic disease preceding the presence of splenic infiltrates, change in spleen weight and detectable abnormal cells in the periphery.

Publications:

Drake, W.P., Schmukler, M., Pendergrast, W.J., Jr., Davis, A., Lichtenfeld, J.L., and Mardiney, M.R., Jr.: Abnormal Profile of Human Nucleolytic Activity as a Test for Cancer. J. Natl. Cancer Inst. 55: 1055-1059, 1975.

Pendergrast, W.J., Jr., Drake, W.P., Schmukler, M., and Mardiney, M.R., Jr.: The Correlation of Abnormal Profiles of Nucleolytic Activity to Cancer. Journal of the National Cancer Institute Monograph "Modulation of Host Immune Resistance in the Prevention and Treatment of Induced Neoplasias ,Second Conference". Michael A. Chirigos, editor, in press, 1976.

ANNUAL REPORT

OFFICE OF THE ASSOCIATE DIRECTOR

CANCER THERAPY EVALUATION PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

The Cancer Therapy Evaluation Program is responsible for: 1) coordinating the introduction of anticancer agents into clinical trials (Investigational Drug Branch (IDB); 2) monitoring the evaluation of such agents by investigators in various clinical cooperative groups, study groups, and independent investigators (IDB and the Clinical Investigations Branch (CIB)); and 3) integrating therapeutic modalities under investigation with other established therapeutic modalities in the treatment of advanced and early cancer of various sites (Combined Modality Branch). Many of these trials are carried out in the clinical cooperative groups which are monitored by the Clinical Investigations Branch, and by contract groups which are monitored by project officers of the Cancer Therapy Evaluation Program (CTEP) or by individuals in other areas of the Division of Cancer Treatment in collaboration with the Associate Director, CTEP.

All the activities of the three branches in the CTEP are integrated by the Associate Director, CTEP, for the purposes of 1) updating information on cancer treatment both from the standpoint of drugs and modalities, and 2) developing strategies for the treatment of various neoplastic diseases according to the leads arising from ongoing clinical trials. The availability of current information on a variety of investigational therapeutic approaches, coupled with extensive monitoring of clinical trials, places the Office of the Associate Director, CTEP, in an ideal position to stimulate therapeutic research along avenues believed most productive. Both contract and grant programs aid in carrying out this research. The disease-oriented contract- and grant-supported programs are described under the activities of the Combined Modality and the Clinical Investigations Branch, respectively. The drug-oriented contracts are described in the report of the Investigational Drug Branch. Only the coordinating efforts under the immediate direction of the Associate Director are dealt with here.

1. Disease-Oriented Strategy

Personnel from the three branches and occasionally elsewhere within the Division of Cancer Treatment serve as disease-oriented coordinators (Table 1). One or more people are involved in monitoring various aspects of a disease and the activities sponsored by the NCI. In general, an interested representative from the Investigational Drug Branch forms part of a team of coordinators. Usually the coordinator(s) is project officer on contracts dealing with the respective disease, and attends all meetings of the contract-related group as well as meetings pertinent to his area, such as various task forces,

disease-oriented committees within cooperative groups, intramural programs, etc. Coordinators are then in ideal positions to serve as reviewers for protocol studies and to help develop strategy outlines.

Flow charts of disease-oriented treatment strategies have been developed for head and neck cancer, lung cancer, small cell carcinoma of the lung, breast cancer, ovarian cancer, colorectum cancer, stomach cancer, melanoma, uterine cancer, and endometrial cancer. Flow charts for testicular cancer and prostate cancer are in the review stage. These documents will be periodically revised to reflect evolving therapeutic trends and questions.

Cooperative group discussions have been instrumental in cross-fertilizing work done by various cooperative groups and individual investigators in specific diseases: lung cancer, gastrointestinal cancer, gynecological cancer, genitourinary cancer, breast cancer, and head and neck cancer. The Head and Neck Cancer Discussion was held on March 25, with guests from Brussels (Dr. A. Jortay, EORTC Secretary for the Head and Neck Group) and from London (Mr. Peter Clifford and Dr. Leonard Price from the Royal Marsden Hospital). Similar sessions are planned in May and June for non-Hodgkin's lymphoma, acute leukemia, and childhood tumors. These sessions have generated great interest among various clinical investigators, which will undoubtedly lead to a more thoughtful and coordinated design of clinical protocols.

Finally, through these meetings the foundations will be laid for the adoption of common criteria of entry, staging, and assessment of response. Master protocols are in preparation for eventual publication and dissemination, which will serve as guidelines for disease-oriented protocols, whether for adjuvant therapy or advanced disease therapy. Conforming to classification and staging of cancer by site, end-results reporting set by the American Joint Committee will be the least common denominator. Encouragement will be given to adoption of common report forms, by disease, which will be a common denominator as a point of departure.

II. Disease-Oriented Studies

1. Phase II/III. All disease areas are included into broad Phase II/III contracts which have been awarded to five major cancer institutions (Table 2). The studies may be complementary to cooperative group or other contract group studies or they may be specialized studies only suitable for single institutions (such as trials of ablative procedures versus drugs in breast cancer). An advantage of this resource of Phase II/III studies is its flexibility. Integration of hyperalimentation into treatment protocols, a detailed analysis of prognostic factors in osteogenic sarcoma, results of adjuvant treatment, and quick incorporation of new drugs into Phase II studies are among products of this flexibility to involve major institutions in studies of a particular therapeutic aspect of a disease. The critical interrelation of modality and disease-oriented thrusts at the Phase II and other levels of clinical testing were described in the FY 1975 annual report.

Table 1. Disease Coordinators

BREAST	Drs. Handelsman, Davis, Muggia, Rozencweig
LYMPHOMA	Dr. Davis
LEUKEMIA	Dr. Jacobs
LUNG	Drs. McGuire, Handelsman, Muggia, Rozencweig, Legha
OVARY	Drs. Young*, McGuire*, Wasserman
GYNECOLOGY	Drs. McGuire*, Wasserman
MELANOMA	Drs. R. Fisher*, Muggia, Jacobs
SARCOMA	Dr. Jacobs
HEAD & NECK	Dr. Chretien**
GI	Drs. Handelsman, Muggia
PROSTATE	Drs. Ihde*, Handelsman, Jacobs
BLADDER, KIDNEY	Drs. Anderson*, McGuire*, Wasserman
TESTIS	Drs. Handelsman, Jacobs
CHILDHOOD TUMORS	Dr. Jacobs
BRAIN	Dr. Walker

*Medicine Branch, COP, DCT **Surgery Branch, COP, DCT

Table 2. Phase II-III Disseminated Tumor Contracts*

M.D. Anderson Hospital and Tumor Institute	Gerald P. Bodey, M.D.
Memorial Hospital	Irwin Krakoff, M.D.
Mayo Clinic	John Edmonson, M.D.
Sidney Farber Cancer Center**	Emil Frei, M.D.
Wayne State University**	Lawrence Baker, M.D.

*Project Officers: Harry Handelsman, D.O., Franco M. Muggia, M.D.
**To be awarded

2. Lung Cancer. The Working Party for Therapy of Lung Cancer, Veterans Administration Lung Group, and the Veterans Administration Surgical Adjuvant Group (contract-supported), as well as four broad multimodality cooperative groups (Cancer and Leukemia Group B, Eastern Cooperative Oncology Group, Southeastern Cancer Study Group, and Southwestern Cancer Study Group), and one specialized cooperative cooperative group (Radiotherapy Oncology Group) constitute the resources for lung cancer research.

3. Large Bowel Cancer. Additional Phase II work is supported by contract (Mayo Clinic--Dr. Charles Moertel, Principal Investigator). This contract has been instrumental in seeking new active drugs in this disease. Adjuvant and other studies are supported by contract in the Gastrointestinal Tumor Study Group; the broad multimodality groups and the RTOG are actively engaged in this area. Studies are also ongoing in the VASAG and in England (Leeds).

4. Breast Cancer. Similarly, studies monitored by our Program includes an additional effort in Phase II supported by contracts (Mayo Clinic--Dr. D. Ahmann, Principal Investigator; Roswell Park Memorial Institute--Dr. T. Dao, Principal Investigator; Albany--Dr. Sponzo). The broad multimodality cooperative groups as well as the Cooperative Breast Cancer Group and the National Breast Surgical Adjuvant Program are other resources. Finally, Dr. Gianni Bonadonna, Italy, has been supported by contract for adjuvant and advanced disease studies.

5. Pancreatic Cancer. Studies in treatment of pancreatic cancer, monitored by the CTEP include the Gastrointestinal Tumor Study Group (contract) and three multimodality groups (SEG, SWOG, and ECOG).

6. Ovarian Cancer. Additional studies are proceeding under Phase II contracts in the area of ovarian cancer (M.D. Anderson--Dr. J. Smith, Principal Investigator; Mt. Sinai--Dr. H. Bruckner, Principal Investigator). The broad multimodality groups (SEG, SWOG, and ECOG) as well as the specialized cooperative groups (RTOG and the Gynecologic Oncology Group) are actively working in this area. A new series of contracts will soon be initiated to study early stage ovarian cancer with adjuvant chemotherapy.

7. Stomach Cancer. The major effort in treatment research in stomach cancer is carried out by the Gastrointestinal Tumor Study Group. Other studies are ongoing in the three broad multimodality groups (ECOG, SEG, SWOG).

8. Brain Tumors. Brain tumor studies are performed by the Brain Tumor Study Group, and also by ECOG, SEG, SWOG, and RTOG.

9. Malignant Melanoma. Studies in treatment of malignant melanoma are carried out by contracts with the WHO Melanoma Study Group, Dr. U. Veronesi in Italy, and by ECOG, SEG, SWOG, and RTOG.

10. Childhood Tumors. The major effort in treatment research in childhood tumors is by the Children's Cancer Study Group. ECOG, CALGB, SEG, and

ALGB also have ongoing studies in this area. Intergroup efforts have been established in Wilms tumor and rhabdomyosarcoma.

11. Adult Leukemia. Studies are ongoing in cooperative groups (CALGB, ECOG, SEG, SWOG).

12. Childhood Leukemia. Studies in this disease have been carried out primarily by the Children's Cancer Study Group and the SWOG's pediatric division, and the CALGB.

13. Lymphomas. The multimodal cooperative groups (CALGB, ECOG, SEG, SWOG) all have studies in malignant lymphomas, all stages. Early stages are included in RTOG studies in joint studies with ECOG.

III. Clinical Trials Data Center

A major function of the CTEP has been to be a central repository of information on clinical trials in cancer. Because of centralization of major contract and grant supported treatment thrust under one program, the opportunity has been provided to develop a data center of all ongoing treatment-related research. Expansion for inclusion of activities not usually in the scope of the CTEP is planned for the future.

The Data Center has been set up by close interfacing with the International Cancer Research Data Bank and development of a system for including all clinical protocols in a registry to be published by the ICRDB yearly. Updating of such a registry and means of abstracting of clinical protocols have been worked out by Dr. T. Wasserman, Special Assistant to the AD in collaboration with Mr. Richard Amacher of the Office of the Director, NCI, the ICRDB, and the Smithsonian Scientific Information Exchange. This ongoing project will have available the results of protocol studies within the cooperative groups, contract-supported studies, intramural NCI studies, and other related activities (such as the European Organization for Research on Treatment in Cancer (EORTC), Swiss Oncology groups, Cancer Center studies, and other grant-supported studies). Centralization of this information will allow for eventual computerization of the data. Access to online ICRDB registry and Cancerline completes the facilities within the Office of the AD to serve as data center for the DCT.

IV. Training Functions

The Office of the Associate Director is concerned with training of staff associates and visiting fellows and associates in: 1) relevant aspects of preclinical drug development, antitumor animal systems, and drug action; 2) means of introduction of drugs into clinical trials; 3) design and execution of clinical trials; 4) clinical pharmacology data in antineoplastic drugs; and 5) other medical aspects of neoplasia. These educational experiences enable the staff to adequately monitor activities within the Branches. Two-year associates have the opportunity to supplement their experience with a six-month clinical rotation at the NCI-VA Medical Oncology Branch or the

Baltimore Cancer Research Center. The first year of training is devoted to acquiring familiarity with the drug development program, screening systems, preclinical toxicity, pharmacology, and drug regulatory affairs. The trainees provide drug information to enquiring physicians by telephone on a rotating basis. During the second year the trainees are allowed choices in assignments--laboratory or clinical (clinical option may be taken as the first or second year) or CTEP. In CTEP, the second year trainee may act as consultant in drug information and monitor of disease-oriented studies. Special projects are assigned which may include collaborative studies with laboratories or clinical units of other division or programs in the DCT. A series of seminars were presented by staff associates and visiting fellows, which included in-depth reviews of anticancer drugs.

V. Collaborative Efforts with Other Countries

The CTEP has continued participation in the U.S. - U.S.S.R. and U.S. - Japan agreements for cooperation in cancer treatment research. Other collaborative efforts are ongoing in England and Italy, by contract. Collaboration is planned for projects in Tunisia and South America, the latter through cooperative effort with the Pan American Health Organization.

VI. Publications

1. Siegel, S.A., and Muggia, F.M.: Treatment of pancreatic cholera. New Engl. J. Med. 293:198, 1975.

2. Wasserman, T.H., Carter, S.K., and Carbone, P.P.: Clinical experience with the nitrosoureas. Proc. XIth Internat. Cancer Cong. Exerpta Medica (No. 353) 5:274-279, 1975.

3. Carter, S.K., and Wasserman, T.H.: The chemotherapy of urologic cancer. Cancer 36:729-748, 1975

4. Perloff, M., and Muggia, F.M.: Carcinoma of the thyroid gland associated with systemic amyloidosis and the nephrotic syndrome. N.Y. State J. Med. 75:1770-1771, 1975.

5. Wasserman, T.H., Comis, R.L., Goldsmith, M., Handelsman, H., Penta, J.S., Slavik, M., Soper, W.T., and Carter, S.K.: Tabular analysis of clinical chemotherapy of solid tumors. Cancer Chemother. Rep. (Part 3) 6(2):299-419, 1975.

6. Wasserman, T.H., Slavik, M., and Carter, S.K.: Clinical comparison of the nitrosoureas. Cancer 36:1258-1268, 1975.

7. Carter, S.K., and Wasserman, T.H.: The interaction of experimental and clinical studies in combined modality treatment. Cancer Chemother. Rep. In press, 1976.

8. Muggia, F.M., Rosen, S.W., Weintraub, B.D., and Hansen, H.H.: Ectopic placental proteins in nontrophoblastic tumors - Serial measurements following chemotherapy. Cancer 36:1327-1337, 1975.

9. Ghossein, H.A., Bosworth, J.L., Stacey, R.T., Muggia, F.M., Krishnaswamy, V.: Radiation-related eosirophilia. Radiology 117(2):413-417, 1975.

10. Von Hoff, D.C., Slavik, M., and Muggia, F.M.: Allergic reactions to cis-platinum. Lancet 1:90, 1976.

11. Von Hoff, D.D., Slavik, M., Muggia, F.M.: 5-azacytidine - a new anti-cancer agent with effectiveress in acute myelogenous leukemia. Ann. Int. Med. In press, 1976.

12. Von Hoff, D.D., Slavik, M., Muggia, F.M.: Incidence of drug deaths secondary to high-dose methotrexate. Cancer Chemother. Rep. In press, 1976.

13. Muggia, F.M., Hansen, H.H., Chervu, L.R.: Diagnosis in metastatic sites. Seminars in Oncology In press, 1976.

VII. Presentations

1. Wasserman, T.: Summary on new anticancer drugs. National Cancer Institute of Canada, Clinical Trials Group, Toronto, Canada, September 10, 1975.

2. Muggia, F.M.: Cell kinetics of acute leukemia. University of Kansas Symposium. Kansas City, Kansas, September 29, 1975.

3. Muggia, F.M.: Kinetic approaches to drug therapy. University of Kansas Symposium. Kansas City, Kansas, September 29, 1975.

4. Muggia, F.M.: Report on NCI organization and disease-oriented discussion groups, Southeast Oncology Group Meeting, Houston, Texas, October 7, 1975

5. Wasserman, T.: Chemotherapy of prostatic cancer. Coperative Urological Radiotherapy Research Group, October 13, 1975, San Francisco, California.

6. Muggia, F.M.: Report on disease-oriented discussion groups. Eastern Cooperative Oncology Group Meeting, Rochester, N.Y., November 18, 1975.

7. Wasserman, T.: CCNU in cancer therapy. FDA Oncologic Drug Advisory Committee, November 20, 1975, Rockville, Maryland.

8. Muggia, F.M.: Report on the Gynecology Group Discussion on December 3, 1975. Gynecologic Oncology Group Meeting, Key Biscayne, Florida, January 29, 1976.

9. Wasserman, T.: Nitrosoureas clinical toxicity. DCT New Drug Seminar, December 15, 1975, Washington, D.C.

10. Muggia, F.M.: Review of adjuvant studies in lung cancer. Meeting of the Executive Committee, Working Party for Therapy of Lung Cancer, Bethesda, Md., January 5, 1976.

11. Muggia, F.M.: Report on the Breast Cancer Group Discussion Meeting. Breast Cancer Task Force Treatment Committee Meeting, Bethesda, Md., January 7, 1976.

12. Wasserman, T.: Nitrosoureas. U.S. - Japan Second Annual Meeting on Chemotherapy, February 6, 1976, Kona, Hawaii.

13. Wasserman, T.: Bleomycin in combination chemotherapy regimens for malignant lymphomas. U.S. - Japan Second Annual Meeting on Chemotherapy, Vebruary 6, 1976, Kona, Hawaii.

14. Muggia, F.M.: Clinical assessment of anticancer drugs. Pharmacology Department Conference, George Washington University School of Medicine, Washington, D.C., February 13, 1976.

15. Wasserman, T.: Cooperative clinical trials in the U.S. National Cancer Institute of Canada Clinical Trials Meeting, Winnepeg, Canada, March 2, 1976.

16. Muggia, F.M.: Current studies in colorectal cancer. Mini-Symposium on Treatment, National Large Bowel Cancer Project Meeting. Houston, Texas, March 5, 1976.

17. Muggia, F.M.: Current protocols in head and neck cancer. Head and Neck Cooperative Group Discussion Meeting, Bethesda, Maryland, March 25, 1976.

18. Muggia, F.M.: Lung cancer: 1. General prognostic factors. 2. Lung cancer studies. 3. Methods and means to lung cancer research. U.S. - U.S.S.R. Joint Lung Cancer Program Review, Moscow, U.S.S.R., April 6, 7, 8, 1976.

19. Muggia, F.M.: Changing concepts of chemotherapy in breast cancer. Stamford Hospital Symposium on Breast Cancer. Stamford, Connecticut, April 28, 1976.

20. Muggia, F.M.: Various teaching presentations during May, 1976, while Ward Attending, NCI-VA Medical Oncology Branch, VA Hospital, Washington, D.C.

21. Muggia, F.M.: Problems with Phase II clinical trials in cancer patients. Workshop on Cancer Chemotherapy, Perugia, Italy, June 30, 1976.

IX. Committee Participation

Franco M. Muggia, M.D.:

Vice-chairman, Combined Modality Committee
Member, Decision Network
Member, Cancer Treatment Program Staff

Member, Clinical Advisory Group for Viral Oncology
Member, Data Review Committee
Member, Operating Committee
Member, Drug Evaluation Subcommittee
NCI Coordinator for Chemotherapy, U.S. - Japan Agreement
Chairman, Lung Committee, U.S. - U.S.S.R. Agreement

Todd Wasserman, M.D.:

Member, Working Cadre, National Bladder Organ Site Cancer Project
Executive Secretary, Clinical Trials Review Committee

IX. Conferences Sponsored by the Cancer Therapy Evaluation Program

Cooperative Group Discussion on Lung Cancer, November 13, 1975
Cooperative Group Discussion on Gastrointestinal Cancer, November 14, 1975
New Drug Seminar, December 15-16, 1975
Cooperative Group Discussion on Genitourinary Cancer, December 17, 1975
Cooperative Group Discussion on Breast Cancer, December 18, 1975
Osteogenic Sarcoma Meeting, January 29-30, 1976
Conference on Epidemiology of Hodgkin's Disease: Dr. James Fenelly (Ireland), February 2, 1976
Conference on Metabolism of Streptozotocin, February 24, 1976
Conference on Cardiotoxicity: Monitoring of Adriamycin Cardiotoxocity with the QRS-Korotkoff Interval, Dr. Antony Greco; Daunomycin Cardiotoxicity, Dr.Daniel Von Hoff, February 24, 1976
Phase I Meeting, March 3, 1976
Conference on Ascorbic Acid and Drug Metabolism, March 3, 1976
Conference on VP16 and VP26, Dr. Franco Cavalli and Dr. Felix Jungi (Switzerland), March 23, 1976
Cooperative Group Discussion on Head and Neck Cancer, March 25, 1976
Conference on High Dose Methotrexate, April 20, 1976
Conference on Hormone Receptors in Breast Cancer, Dr. J.C. Heuson (Jules Bordet Institute), April 27, 1976
Conference on L-Asparaginase:A Review, Dr. William Hrushesky, May 11, 1976
Cooperative Group Discussion on Lymphoma, May 18, 1976
Cooperative Group Discussion on Adult Leukemia, May 26, 1976
New Drug Liaison Meeting, May 26-28, 1976
Cooperative Group Discussion Meeting on Pediatric Oncology, June 24, 1976

(All the above meetings and conferences were held at NIH, Bethesda, Maryland)

CLINICAL INVESTIGATIONS BRANCH

The Clinical Investigations Branch, under the direction of Dr. Raul Mercado, Jr., is responsible for the administrative and scientific management of the Clinical Cooperative Groups. The major multi-disciplinary, multi-disease groups are:

Acute Leukemia Group B
Children's Cancer Study Group
Eastern Cooperative Oncology Group
Southeastern Cancer Study Group
Southwest Oncology Group

Other major specialized groups are:

Cooperative Breast Cancer Group
Gynecologic Oncology Group
National Wilm's Tumor Study Group
Primary Breast Cancer Therapy Group
Radiation Therapy Oncology Group
Veterans Administration Uro-Oncology Group

Special projects supported by the Clinical Investigations Branch are:

Polycythemia Vera Study Group
Malignant Melanoma Group
Lymphoma Reference Center
Radiologic Physics Center

The Cooperative Groups conduct studies to determine the dose-toxicity relationships of potentially useful new chemotherapeutic agents in human malignancies (Phase I Studies) in a limited number of institutions. They conduct extensive studies of new anti-cancer drugs in a wide spectrum of human malignancies to determine the response rates and toxicities of potentially useful new agents (Phase II Studies). These studies are a major source of information for the National Cancer Institute in fulfilling the requirements of the Food and Drug Administration concerning new agents. During this year the major groups have taken steps to expand their research activities for prospective comparative studies (Phase III). Emphasis has been placed on planned sequential studies to follow a logical experimental approach, each succeeding study usually being derived from information from preceding studies and the data of other groups and investigators. Increased emphasis is placed on the use of clinical pharmacology and pharmacokinetics as background.

Research activities of the Cooperative Groups are increasingly directed to the studies of patients with early disease, utilizing combination chemotherapy, combined modality therapy, and adjuvant or prophylactic therapy. To this end supplemental funds are being made available to the groups to expedite the further development of Specific Disease Committees and Specialty

Committees for involvement of and recruitment of all the major disciplines into group efforts.

Additionally, increased support has been provided to develop and ensure more accurate and efficient methods of record keeping, data storage, data retrieval and evaluating treatment programs.

In the interest of fostering improved inter-group cooperation, Disease-Oriented Discussion Groups have been organized. At the meetings of these groups representatives of the Cooperative Groups exchange information regarding a particular cancer or site. Discussions during these meetings have indicated the desirability of developing a uniform system for reporting data. Another likely outcome is forward planning and coordination of future studies.

During this year meetings of the following Discussion Groups have been held:

Lung Cancer
Breast Cancer
Gastrointestinal Cancer
Gynecologic Cancer
Genito-Urinary Cancer
Head and Neck Cancer

Other meetings proposed for this year include:

Non-Hodgkins Lymphoma
Adult Leukemia
Pediatric Solid Tumors

The Clinical Investigations Branch is responsible for the review of any proposed new studies from the Cooperative Groups. This is required prior to filing of the studies with the Food and Drug Administration in order to obtain investigational drugs. Dr. Edwin Jacobs has joined the CIB recently and has the primary responsibility for coordination of the review process. Review is performed primarily by the professional staff of the Cancer Therapy Evaluation Program and other branches of the Division of Cancer Treatment as needed. Outside consultants are used if their particular area of expertise is considered necessary for the review. The lag between conception and implementation of a new protocol has been significantly reduced by primary reliance on intramural scientists for the review process.

Though the Clinical Cooperative Groups are funded primarily to carry out therapeutic research, they have become an effective mechanism for dissemination of information concerning optimal forms of therapy for different cancers. It is anticipated that during this year, funds will become available to the Cooperative Groups from the Division of Cancer Control and Rehabilitation to expand their efforts in control activities by encouraging the participation of community physicians in the clinical trials efforts of the Cooperative Groups.

The Clinical Investigations Branch, with the Cancer Clinical Investigation Review Committee, sponsors scientific symposia relating to subjects of interest to investigators involved in clinical trials. A symposium on Epidemiology of Cancer was held in Boston in October 1975. A symposium on Brain Tumors was held in Atlanta in February 1976. Both of these meetings were open to the public and were attended by a large number of clinical investigators.

Presentations:

1. Mercado, R.: The Role of the Clinical Investigations Branch in Cancer Research. American Association of Physicists in Medicine, San Antonio, Texas, August 6, 1975

2. Mercado, R.: Cancer Clinical Cooperative Groups. New England Radiation Oncology Society, Boston, Massachusetts, March 26, 1976.

3. Mercado, R.: Extemporaneous remarks at most meetings of the Cooperative Groups.

COMBINED MODALITY BRANCH

This branch continues to monitor Phase II and Phase III contract activities within the Division of Cancer Treatment in the area of solid tumors.

The status of current contract supported studies is reviewed under the respective groups. The major thrust of the groups has been in combined modality studies following leads provided in the treatment of advanced disease.

The Working Party for the Therapy of Lung Cancer

This group conducts single agent and combined modality studies in patients with lung cancer.

Dr. Ronald G. Vincent is the current Group Chairman. Plans to expand the group from its present six institutions to facilitate patient accrual and expedite completion of group studies are awaiting issuance of a new RFP.

Current protocols are operative for limited and extensive disease patients with stratifications to three cell types; small-cell, epidermoid and a combined stratification for adeno and large-cell carcinoma.

A protocol comparing CTX vs COMB (CTX, MeCCNU, VCR, Bleo) in patients with extensive epidermoid carcinoma has been completed and is being prepared for publication.

A protocol comparing short vs long course radiotherapy of regional epidermoid carcinoma has also been completed and final analysis awaits follow-up of accrued cases.

Testicular Tumor Study Group

This group was formed primarily for a study of radiotherapy vs chemotherapy vs the combination of both in Stage II testicular carcinoma.

The contracts for the conduct of a Stage II disease protocol expired on December 31, 1975 with no significant patient accrual into the study. The two factors most responsible for the poor accrual was the increasing success of new combination chemotherapy in this disease coupled with the decreased numbers of patients found to have Stage II disease as a result of better diagnostic technique and more thorough staging procedures.

The group contributed greatly to discussions on the changing role of chemotherapy in this disease.

As a result of this evolving situation, a staff decision was made to phase out the contracts and for the present encourage generation of Stage III protocols via the cooperative groups, and to defer the development of additional Stage I and II protocols.

Gastrointestinal Tumor Study Group

This group has designed protocols to investigate pancreatic, stomach and colorectal carcinomas with particular emphasis on minimal residual disease and the adjuvant setting.

Three gastric cancer and three pancreatic cancer studies are operational investigating surgical adjuvant therapy, locally unresectable disease and advanced disease.

A study of single agents in advanced pancreatic disease has been completed and appears as an abstract of the AACR.

Two colorectal studies are ongoing to investigate surgical adjuvant therapy of high-risk patient groups. Both of these studies share common arms with group studies by the RTOG and ECOG.

Phase II - Phase III Studies in Patients with Disseminated Solid Tumors

Effective June 30, 1975 contracts for Phase II and Phase III studies in patients with disseminated solid tumors were let to the Mayo Clinic, Sloan Kettering Memorial Hospital, and M. D. Anderson Hospital.

A wide range of Phase II and III trials involving a multitude of tumor types are being conducted by the three institutions, i.e., lung, breast, colon, sarcoma, melanoma, head and neck, ovary, cervix, thyroid, kidney, gastric, and testicular carcinomas.

In general the case accrual to these studies has been excellent and it is expected that the number of studies will continue to expand.

Phase II - Phase III Breast Contracts

Mayo Clinic - CM 53772

This contract was designed to support prospective randomized controlled studies using combination chemotherapy in the treatment of breast cancer and a current study of chemotherapy as an adjuvant to oophorectomy in patients with breast cancer.

Chemotherapy Protocols include:

1. ADM + Alkeran vs 5-FU + CTX + Prednisone

2. Galactitol vs ICRF 159

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INVESTIGATIONAL DRUG BRANCH

In the past fiscal year the Investigational Drug Branch (IDB) functioned in four major directions: (a) chemotherapy evaluation, (b) clinical pharmacology, (c) regulatory aspects, and (d) distribution of investigational drugs. Organizationally the Branch consists of the Office of the Chief and the Drug Liaison and Distribution Section. The professional staff of the Branch includes the physican, pharmacologist, pharmacist and three staff associate physicians who spend the first year of their Cancer Therapy Evaluation Program training in the Investigational Drug Branch. In addition, one visiting fellow joined the Branch in the International Exchange Program.

The coordination of all the functions of the Investigational Drug Branch is done by the office of the Chief, which in addition is responsible for chemotherapy evaluation and liaison with the cooperative groups, evaluation and approval of investigational drug protocols submitted by investigators conducting independent studies and training of the staff associates in the drug oriented part of the CTEP. In addition, this office is responsible for monitoring and management of Phase I contracts awarded to seven institutions. The Branch also participated in a substantial number of international activities. The regulatory function and distribution of investigational drugs throughout the United States and the world is provided by the Drug Liaison and Distribution Section, IDB. Following is the outline of the various functions of the Investigational Drug Branch.

Monitoring of clinical studies and chemotherapy evaluation.

Phase I Studies: Nine investigational drugs sponsored by the DCT were studies in Phase I trials:

Gallium Nitrate	NSC 15200
Galactitol	NSC 132313
Anguidine	NSC 141537
Aminothiadiazol	NSC 4728
Dichlorallyl lawsome	NSC 126771
MeCCNU (i.v.)	NSC 95441
Pyrazofurin	NSC 143095
Maytansine	NSC 153858
Neocarzinostatin	NSC 157365

The Phase I studies were conducted by members of the Phase I Working Group, which consists of clinical investigators from four branches of the NCI Intramural Program, clinical investigators from seven institutions awarded the Phase I contract and five institution conducting Phase I studies without any financial support. Eighteen Phase I studies were conducted and seven of them completed. Two Phase I drugs entered Phase II studies. Phar-

macokinetic studies in man were included in three Phase I studies. The coordination of Phase I studies is accomplished by ad hoc telephone conferences and by Phase I Working Group Meetings approximately three times a year. Detailed information on the current activities of the Phase I Working Group Meeting and is available from the Investigational Drug Branch.

Phase II Studies: Among the drugs evaluated in Phase II-III studies are:

Daunomycin	NSC 82151
5-azacytidine	NSC 102816
Hexamethylmelamine	NSC 13875
Cis-platinum-diamminedichloride	NSC 119875
Isophosphamide	NSC 109724
VM-26	NSC 122819
VP-16	NSC 141340
ICRF 159	NSC 129943
Yoshi 864	NSC 102627
Chromomycin A_3	NSC 58514
Cyclocytidine	NSC 145668
Baker's Antifol	NSC 139105
Cytembena	NSC 104801
Piperazinedione	NSC 135758
Diglycoaldehyde	NSC 118994
Ftorafur	NSC 198958

The drugs for which NDA has been filed or considered include:

BCNU	NSC 409962
CCNU	NSC 79037
MeCCNU	NSC 95441
L-asparaginase	NSC 109229
Daunomycin	NSC 82151
Streptozotocin	NSC 85998
Hexamethylmelamine	NSC 13875

The update and exchange of information on Phase II-III clinical studies is done by the New Drug Liaison Meeting, which is planned for May 27-28. Minutes of this meeting are available from the Investigational Drug Branch.

Liaison with Cooperative Groups and Phase II Contracts: The members of the IDB professional staff attend more than 30 cooperative group meetings in a year. They actively participate also at the Phase II-III contractors meetings as well as in the disease oriented discussion and coordination meetings organized by the CTEP.

Evaluation of Independent Investigator Protocols: Within the last year the number of protocols submitted by independent investigators

drastically increased from an average of 15 protocols a week to 100 protocols a week. This was mainly caused by the fact that FDA did not approve NDA's for several investigational drugs (see-drugs NDA) which have been pending for several years. Recently a mechanism has been worked out to diminish the workload by development of common protocol (guidelines) for seven of these drugs, in order to decrease the tremendous workload for the Branch.

Training of Staff Associates: In the last year three new associates joined the training program of the Branch. This consists of the drug-disease oriented program in cancer chemotherapy and clinical pharmacology of anticancer drugs. In this program, in addition to the daily educational activities, series of lectures on drug development, screening, mode of action, clinical pharmacology, toxicology and chemotherapy of leukemias, lymphomas, and solid tumors were given by the members of the CTEP, Drug Development, and Experimental Therapeutics staff. The program will be further expanded.

Feedback to Drug Development Program: There has been a continuous feedback to the Drug Development Program through several mechanisms (operating committee, drug evaluation committee and decision network committee). In addition, critical analysis of several parts of the Drug Development Program has been undertaken by the Investigational Drug Branch which is resulting in several studies such as correlation of clinical and experimental animal antitumor activity of investigational drugs, correlation of clinical and experimental animal toxicity, setting up a running talley for clinical evaluation of Phase II-III clinical studies, critical evaluation of investigational anticancer drugs from foreign resources, etc.

Drug Liaison and Distribution Section

Food and Drug Administration Activities

Investigational New Drug Notices: There were seven investigational new drug (IND) notices which were prepared and submitted to either the Bureau of Drugs or Bureau of Biologics, Food and Drug Administration (FDA). These drugs are:

Hycanthone	NSC 142982
Pyrazofurin	NSC 143095
Neocarzinostatin	NSC 157365
Thiadiazole	NSC 4728
Maytansine	NSC 153858
Fluorodopan	NSC 74754
Merieux C. Parvum	NSC 197213

Hycanthone has had prior clinical use in the treatment of human schistosomiasis. It is of interest as a potential oncolytic agent

because it is an intercalator of DNA, displays antitumor activity in animals, and demonstrates little myelosuppression in animals. Pyrazofurin, a congener of 6-azauridine, shows antitumor activity in a number of experimental tumor systems. This drug has already undergone previous clinical trials under the IND sponsorship of Eli Lilly and Co., and that company's IND was cross-referenced by NCI. Neocarzinostatin is a polypeptide which was developed and put into clinical trials by the Japanese. It is reported to be active in AML and in pancreatic disease. This Section worked closely with Bristol Laboratories in the preparation and submission of this IND. Thiadiazole underwent limited clinical trial in the late 1950s, but these had to be curtailed because of marked uric acid production. With the development of allopurinol, it is now intended to study the potential antitumor activity of this drug in humans. Maytansine, a plant product, belongs to the ansa macrolide class. It has demonstrated antitumor activity in animals at doses of micrograms per kilogram body weight against P388 leukemia and B16 melanoma. Fluorodopan, a uracil mustard analogue, is one of three exchange drugs which the Soviet Union provided to the NCI. In that country, this drug is reported to have activity in lymphosarcoma, reticulum cell sarcoma, and fibrosarcoma. Merieux C. Parvum is obtained from the Institute Merieux, Lyon, France. This product is a suspension of heat killed corynebacterium parvum, and is thought to be a potent stimulant of the RES. Early clinical trials have been carried out in France.

Immunology Liaison: The Section continues to provide a liaison between the Bureau of Biologics, Food and Drug Administration, and the clinical cooperative study groups regarding regulatory requirements for cooperative study group trials which utilize immunotherapy. Much time and effort has been expended to satisfy FDA regulatory requirements by obtaining institutional review committee approval and patient consent forms from member institutions of the cooperative study groups.

Drug Distribution Activities: During the past year the Section initiated the development of a clinical drug order verification procedure. Contract support from the Value Engineering Company was obtained, and a systems analysis of the drug distribution procedures was undertaken. The objective of this system is to insure that investigational anticancer drugs are distributed efficiently and in conformance with the Food and Drug Administration requirements.

The initial order verification procedure involves comparing incoming drug orders against hard copy files to ascertain the correctness of the investigator registration status, the protocol number, the drug, and dosage form. Any discrepancies are brought to the attention of the investigator for correction. At the present time

the hard copy files are in need of updating. Once this is accomplished, the files will be computerized, and the order certification procedure will be automated.

Scientific Activities

High-dose Methotrexate plus Citrovorum Factor Rescue: A study of the incidence of drug-associated fatalities secondary to administration of high-dose methotrexate plus citrovorum factor rescue has begun. Preliminary analysis of 498 patients treated indicates 29 fatalities, an incidence of 6%. The majority of these fatalities appears to be due to severe myelosuppression with sepsis or hemorrhage and, to a lesser degree, another cause of fatality is renal failure. This study is continuing.

Clinical Pharmacology of Oncolytic Agents: The Section has taken steps to collect and disseminate clinical pharmacology data for investigational oncolytic agents. Reviews of VP-16 (NSC 141540) and ICRF-159 (NSC 129943) have been prepared and disseminated to various cooperative study groups. The clinical pharmacology of 22 other agents is presently under review, and this information will be submitted for publication.

HONORS AND AWARDS

1. Slavik, M. Consultant, FDA Oncology Advisory Committee.

2. Slavik, M. Chairman, Section on Hematologic and Neoplastic Diseases, American Society of Clinical Pharmacology and Experimental Therapeutics.

3. Slavik, M. Member, Reference Panel of American Hospital Formulary Services, ASHP.

4. Penta, J.S. Visiting Faculty Member, Department of Medical Oncology, Mayo Clinic, Rochester, Minnesota.

5. Penta, J.S. Appointed to Membership of Developmental Therapeutics Committee, Southeastern Cancer Study Group.

PUBLICATIONS

1. Goldsmith, M.A., Slavik, M., and Carter, S.K.: Quantitative Prediction of Drug Toxicity in Man from Toxicology in Small and Large Animals. Cancer Research 35:1354-1364, 1975.

2. Slavik, M.: Nucleoside Analogues in Treatment of Neoplastic and Nonneoplastic Diseases. Annals New York Academy of Science 225:266-268, 1975.

3. Slavik, M.: Adriamycin Activity in Genitourinary and Gyneco-

logic Malignancy. Cancer Chemother. Rep. Part 3, Vol. 6 297-303, 1975.

4. Carter, S.K., and Slavik, M.: A Chemotherapist's Perspective on Clinical Trials with Cornebacterium Parvum. Internal Symposium on C. Parvum. Corynebacterium Parvum, edited by B. Halpern, Tenum Publishing Corp., 329-340, 1975.

5. Carter, S.K., Slavik, M., and Wasserman, T.H.: An Overview of Single Agent Testing in Solid Tumors and New Investigational Agents. Cancer Chemother Fundamental Concepts and Recent Advances. Yearbook Medical Publishers, 99-148, 1975.

6. Catalanotto, F.A., Slavik, M., Danilson, D., Keiser, H.R., and Henkin, R.I.: Changes in Preference for NaCL Following Administration of 6-Azauridine and 6-Azauridine Triacetate. Pharmacology and Therapeutic in Dentistry 2:25-32, 1975.

7. Wasserman, T.H., Slavik, M., and Carter, S.K.: Clinical Comparison of the Nitrosoureas. Cancer 36:1258-1268, 1975.

8. Chabner, B., and Slavik, M.: Introduction: Perspectives on High Dose Methotrexate (NSC 740) Therapy. Cancer Chemother. Rep. Part 3, Vol. 6:1-2, 1975.

9. Wasserman, T.H., Comis, R.L., Goldsmith, M., Handelsman, H., Penta, J.S., Slavik, J., Soper, W.T., and Carter, S.K.: Tabular Analysis of the Clinical Chemotherapy of Solid Tumors. Cancer Chemother. Rep. Part 3, Vol. 6:399-419, 1975.

10. Slavik, M.: New Anticancer Drugs Sponsored for Clinical Studies by the National Cancer Institute. Kanser 5:1-24, 1975.

11. Von Hoff, D.D., Slavik, M., and Muggia, F.M.: Allergic Reactions to Cis-platinum. Lancet 1:90, 1976.

12. Von Hoff, D.D., and Slavik, J.: Daunomycin Cardiotoxicity. Clinical Pharmacology Therapeutics Vol. 19:117-118, (abstract) 1976.

13. Legha, S., Slavik, M., and Carter, S.K.: Hexamethylmelamine: An Evaluation of its Role in the Therapy of Cancer. Cancer (in press).

14. Von Hoff, D.D., and Slavik, M.: 5-azacytidine - A New Anticancer Drug with Significant Activity in Acute Myeloblastic Leukemia. Advances in Pharmacology and Chemotherapy (in press).

15. Von Hoff, D.D., Slavik, M., and Muggia, F.M.: 5-azacytidine - A New Anticancer Drug with Effectiveness in Acute Myelogenous

Leukemia. Annals of Internal Medicine (in press).

16. Von Hoff, D.D., Penta, J.S., Hellman, L.J., and Slavik, J.: The Incidence of Drug Related Death Secondary to High Dose Methotrexate (NSC 740) and Citrovorum Factor (NSC 3590) Administration. Cancer Treat. Rep. (in press).

17. Slavik, M.: Clinical Studies with DTIC (NSC 45388) in Various Malignancies. Cancer Treat. Rep. 60: 213-214, 1976.

18. Slavik, M.: Clinical Studies with Nitrosoureas in Solid Tumors. Cancer Treat. Rep. (in press).

19. Carter, S.K., and Slavik, M.: Investigational Drugs Under Study by The National Cancer Institute. Cancer Treat. Rev. (in press).

20. Legha, S., Slavik, M., and Carter, S.K.: Nafoxidine - An Antiestrogen for the Treatment of Breast Cancer. Cancer (in press).

21. Kleinman, L.M., Davignon, J.P., Cradock, J.C., Penta, J.S., and Slavik, M.: Investigational Drug Information on Hexamethylmelamine, Dacarbazine and Carmustine. Drug Intelligence and Clinical Pharmacology 10:48-49, 1976.

22. Penta, J.S.: Overview of Protocols on Clinical Studies of High Dose Methotrexate (NSC-740) with Citrovorum Factor (NSC 3590) Rescue. Cancer Chemother. Rep. Part 3, Vol. 6 (1):7-12, 1975.

23. Von Hoff, D.D., Penta, J., Vilk, P.: Prolonged Intravenous Infusions of 5-azacytidine. Lancet (in press).

24. Hrushesky, W.: Supravenous Fuluorouracil Hyperpigmentation. Cancer Chemother. Rep. (in press).

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26. Hrushesky, W., Muggia, F., and Slavik, M.: Summary of Results with L-asparaginase Therapy. Proceedings ASCO. May 1976.

27. Hrushesky, W.: The Place of L-asparaginase in Cancer Therapy. Proceedings of Annual USPHS Professional Association. (in press).

28. Hrushesky, W.: Unusual Pigmentary Changes Associated with Intravenous Fluorouracil Administration. JAMA (in press).

29. Strauss, M.S.: Piperazinedione. Clinical Brochure, May 1975.

30. Keller, A.M., Handelsman, H., and Slavik, M.: Isophosphamide. Clinical Brochure (in press).

31. Von Hoff, D.D., Handelsman, H., and Slavik, M.: 5-azacytidine (NSC 102816). Clinical Brochure, August 1975.

32. Von Hoff, D.D., and Slavik, M.: Cytembena (NSC 104801). Clinical Brochure, September 1975.

33. Von Hoff, D.D., and Slavik, M.: Daunomycin (NSC 82151). Clinical Brochure, December 1975.

34. Von Hoff, D.D., and Slavik, M.: Etiocholanolone (NSC 50908). Clinical Brochure, September 1975.

35. Von Hoff, D.D., and Slavik, M.: Trimethylcolchicinic Acid. Clinical Brochure, December 1975.

36. Legha, S., and Slavik, M.: Baker's Antifol (NSC 139105). Clinical Brochure, May 1975.

PRESENTATIONS

1. Slavik, M.: Hexamethylmelamine and DTIC. Current Status of Clinical Studies in the United States. Presented at the US - USSR Joint Working Group Meeting. Bethesda, Maryland, April 15, 1975.

2. Slavik, M.: DTIC - Other Studies. Sixth New Drug Seminar. Bethesda, Maryland, April 28, 1975.

3. Slavik, M.: Metallic Compounds in Cancer Chemotherapy - Overview of Clinical Studies. Symposium on Metallic Compounds in Cancer Chemotherapy. Oak Ridge National Laboratory. Oakridge, Tennessee, April 29, 1975.

4. Slavik, J.: Galactitol - An Update on Clinical Studies in the United States. Southwest Oncology Group Meeting. St. Louis, Mo., June 11, 1975.

5. Slavik, M.: What the Phase I Working Group has Accomplished to Date. Phase I Working Group Meeting. Bethesda, Maryland, June 24, 1975.

6. Legha, S.: Analysis of Phase I Clinical Trials of Baker's Antifol (NSC 139105). Presented at the Phase I Working Group Meeting, Bethesda, Maryland, June 24, 1975.

7. Penta, J.S.: Anticancer Drugs. Presented to the School of Nursing, Northern Virginia Community College, Annandale, Virginia, August 18, 1975.

8. Penta, J.S.: Development of New Investigational Oncolytic Agents. Presented to the Department of Medical Oncology, Mayo Clinic, Rochester, Minnesota, September 4, 1975.

9. Penta, J.S.: Integration of Pharmacology into Combination Cancer Chemotherapy. Presented to the Department of Medical Oncology, Mayo Clinic, Rochester, Minnesota, September 4, 1975.

10. Slavik, M.: Recent Advances in Chemotherapy of Cancer. New York Chemical Society, Terrytown, Westchester County, New York, September 19, 1975.

11. Slavik, M.: Clinical Observation and EKG Changes Associated with Adriamycin Therapy. Summation of the Session's Chairman. Oncology-Cardiology Workshop on Adriamycin Cardiotoxicity, Dayton, Ohio, September 24, 1975.

12. Von Hoff, D.D.: ICRF-159, Piperazinedione and Galactitol - Three New Anticancer Compounds. VA Lung Group Meeting, Memphis, Tenn., October 3, 1975.

13. Slavik, M.: D-tetrandrine (NSC 77037). A New Anticancer Drug Available for Phase II Studies. Presented at the Southwest Oncology Group Meeting, Houston, Texas, October 8, 1975.

14. Von Hoff, D.D.: Hexamethylmelamine - An Agent for Adjuvant Use? VA Surgical Adjuvant Group Meeting, San Francisco, Ca., October 12, 1975.

15. Slavik, M.: Overall Concept and Progress Report of Phase I Working Group. Contract Review Combined Modality Committee, Bethesda, Maryland, October 30, 1975.

16. Slavik, M.: Pyrimidine Nucleoside Analogues in Cancer Chemotherapy. Brown University, Providence, Rhode Island, November 3, 1975.

17. Slavik, M.: Advances in Chemotherapy of Breast Cancer. Michigan State University, Saginaw, Michigan, November 6, 1975.

18. Rozencweig, M.: Review on Chemotherapy of Advanced Lung Cancer. Cooperative Group Discussion Meeting on Lung Cancer, Bethesda, Maryland, November 11, 1975.

19. Slavik, M.: New Drugs Available for Phase II Studies. Phase II Contractors Meeting, Bethesda, Maryland, November 12, 1975.

20. Slavik, M.: Experimental Design of Phase I Studies. FDA Advisory Committee. Rockville, Maryland, November 20, 1975.

21. Hrushesky, W.: A Review of the Clinical Utility and Toxicity of L-asparaginase in some 8,000 Patients. Bureau of Biologics, FDA, Bethesda, Maryland, December 12, 1975.

22. Slavik, M.: Clinical Studies with Nitrosoureas in Other Tumors. New Drug Seminar, Washington Hilton, Washington, D.C., December 16, 1975.

23. Rozencweig, M.: Current Cooperative Studies in Bronchogenic Carcinoma. Canadian Lung Cancer Working Party, Winnipeg, Canada, December 19, 1975.

24. Penta, J.S.: Incidence of Drug-Related Fatalities Due to High Dose Methotrexate. Osteogenic Sarcoma Study Group Meeting, Bethesda, Maryland, January 29, 1976.

25. Slavik, M.: New Drugs Available for Phase II Studies on Gynecological Malignancies. GOG Meeting, Key Biscayne, Florida, January 30, 1976.

26. Von Hoff, D.D.: Incidence of Drug Related Death After High Dose Methotrexate Treatment. Workshop on Antimetabolites, New York, New York, February 2, 1976.

27. Penta, J.S.: Clinical Pharmacology of High Dose Methotrexate Plus Citrovorum Factor Rescue. Children's Cancer Study Group, San Francisco, California, February 19, 1976.

28. Penta, J.S.: Clinical Trials with VP-16-213 (NSC 141540) Administered by the Oral Route. Children's Cancer Study Group, San Francisco, California, February 20, 1976.

29. Slavik, M.: Isophosphamide - Current Status and Prospectives. International Conference on Ifosfamide, Atlanta, Georgia, March 2, 1976.

30. Penta, J.S.: The Potential Use of Optical Isomers of ICRF-159 (NSC 129943) for Clinical Trials. Phase I Working Group, Bethesda, Maryland, March 10, 1976.

31. Slavik, M.: Deazauridine - New Drug Available for Phase I Studies. Phase I Working Group Meeting, Bethesda, Maryland, March 10, 1976.

32. Rozencweig, M.: Adenocarcinoma of the Lung. Working Party for Lung, Houston, Texas, March 15, 1976.

33. Von Hoff, D.D.: Daunomycin Cardiotoxicity. Seventh Annual

Meeting of the American Society for Clinical Pharmacology and Therapeutics, Seattle, Washington, March 18, 1976.

34. Rozencweig, M.: Review of Chemotherapy of Head and Neck Cancer. Cooperative Group Discussion Meeting on Head and Neck Cancer. March 25, 1976.

35. Von Hoff, D.D.: Daunomycin Cardiotoxicity, Fifth Seventh Annual Session of the American College of Physicians, Philadelphia, Pennsylvania, April 8, 1976.

36. Slavik, M.: Current Clinical Status of Daunomycin. Working Symposium on Anthracycline Antibiotics, April 22, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 6600-01 IDB

PERIOD COVERED
July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)
Cardiotoxicity of Cancerostatic Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Daniel D. Von Hoff, M.D., Staff Associate, Investigational Drug Branch, Cancer Therapy Evaluation Program, DCT, NCI
Milan Slavik, M.D., Ph.D., Chief, Investigational Drug Branch, Cancer Therapy Evaluation Program, DCT, NCI
Maxwell Layard, Ph.D., Biostatistician, Biometry Branch, Division of Cancer Cause and Prevention, NCI
Franco M. Muggia, M.D., F.A.C.P., Associate Director, Cancer Therapy Evaluation Program, DCT, NCI

COOPERATING UNITS (if any)
Biometry Branch, Division of Cancer Cause and Prevention

LAB/BRANCH
Investigational Drug Branch, Cancer Therapy Evaluation Program, DCT, NCI

SECTION
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INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	.5

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the cardiotoxicity of currently available and investigational anticancer drugs. The anticancer drugs currently under investigation for their carciotoxic effects include the anthracycline analogs.

The ultimate goals of this project will be: 1) to attempt to define relationships between total doses of the drugs and cardiotoxicity; 2) to define the manifestations of the drug-induced cardiotoxicity; and 3) to define a safe dose of the drugs to be used by the treating physician.

PHS-6040
(12-75)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 6601-01 IDB

PERIOD COVERED
July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)
Correlation of Clinical and Antitumor Activity of Single Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Marcel Rozencweig, M.D., Visiting Fellow, Investigational Drug Branch, CTEP, DCT, NCI
Milan Slavik, M.D., Ph.D., Chief, Investigational Drug Branch, CTEP, DCT, NCI
Franco Muggia, M.D., F.A.C.P., Associate Director, Cancer Therapy Evaluation Program, DCT, NCI
John M. Venditti, Chief, Drug Evaluation Branch, DEB, DR & D

COOPERATING UNITS (if any)
Drug Evaluation Branch, DR & D

LAB/BRANCH
Investigational Drug Branch, Cancer Therapy Evaluation Program

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the correlation of the activity in men and experimental animals of single anticancer drugs.

The ultimate goals of this project will be; (1) to define a predictive mathematical model of clinical activity of new anticancer drugs based on animal data; (2) to evaluate the predictive value in man of the present and the future animal tumor models for clinical studies.

PHS-6040
(12-75)

60 anticancer drugs have been introduced in clinical studies so far. The activity of all these drugs has been tested in five mice tumor systems i.e., P 388 leukemia, L1210 leukemia (solid & ascites form), B16 melanoma and Lewis lung carcinoma. For all these drugs, in each of these experimental systems, an ILS has been determined (ILS = Increase in life span of the treated mice as compared to the survival of untreated controls). The clinical activity of these drugs is presently evaluated by various tumor types.

For each of the most frequently investigated human tumors, a mathematical model will be defined according to the clinical and animal data. Hopefully, this model will allow us to predict the activity of investigational drugs in the most frequent tumor types based on ILS found in the previously mentioned experimental tumors. This model will also allow to determine the predictive contribution of new experimental tumors.

A continuous updating of the activity of investigational drugs will take place through a running talley system. This update will permit a permanent control and improvement of the model.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CM 6603-01 IDB

PERIOD COVERED
July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)
Correlation of Clinical and Experimental Animal Toxicity of Anticancer Drugs.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Marcel Rozencweig, M.D., Visiting Fellow, Investigational Drug Branch, CTEP, DCT, NCI
Milan Slavik, M.D., Ph.D., Chief, Investigational Drug Branch, CTEP, DCT, NCI
Franco Muggia, M.D., F.A.C.P., Associate Director, Cancer Therapy Evaluation Program, DCT, NCI
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C.L. Litterst, Ph.D., Senior Staff Fellow, LT, ETP, DCT, NCI

COOPERATING UNITS (if any)
Toxicology Branch

LAB/BRANCH
Investigational Drug Branch

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the correlation of the toxicity in men and experimental animals of single anticancer drugs.

The ultimate goals of this project will be; (1) to define a safe and effective starting dose of anticancer drugs when studied the first time in man; (2) to evaluate the predictive value of animal toxicity data in man.

PHS-6040
(12-75)

All the drugs which have been tested I.V. in men with the following schedules will be analysed: high intermittent dose weekly and daily x 5. Clinical toxicity will be evaluated for all of these drugs.

Values of LD10, LD50 and LD90 will be determined at each of these schedules in rodents. Thorough anatomic examinations will also be performed at each of these lethal dose levels. A mathematical model will be tentatively defined formthe correlation of the clinical and animal data. Hopefully, this model will enable to predict a safe but also an effective dose of investigational drugs in men based on toxicity data in rodents. This study should also show to what extent qualitative toxicology in rodents might be predictive in men.

A continuous updating of the toxicity of investigational drugs will take place through a running talley system. This update will permit a permanent control and improvement of that correlation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 6602-01 IDB

PERIOD COVERED
July 1, 1975 - June 30, 1976

TITLE OF PROJECT (80 characters or less)
Predictive Value in Men of the Nude Mice Experimental System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Marcel Rozencweig, M.D., Visiting Fellow, Investigational Drug Branch, CTEP, DCT, NCI
Alan Keller, M.D., Special Assistant to Chief, Investigational Drug Branch, CTEP, DCT, NCI
Milan Slavik, M.D., Ph.D., Chief, Investigational Drug Branch, CTEP, DCT, NCI
Franco Muggia, M.D., F.A.C.P., Associate Director, Cancer Therapy Evaluation Program, DCT, NCI
R.K. Johnson, Ph.D., Acting Head, Laboratory of Experimental Chemotherapy, Tumor Biology Section, DCT, NCI
R. Osieka, M.D., Guest Worker, Laboratory of Experimental Chemotherapy, Tumor Biology Section, DCT, NCI

COOPERATING UNITS (if any)
NCI - VA

LAB/BRANCH
Investigational Drug Branch

SECTION

INSTITUTE AND LOCATION
National Cancer Institute, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range purpose of this project to study the correlation of the efficacy in men and in xenografts of cytotoxic agents.

The ultimate goal of this project is to determine to what extent this experimental system must predict the effecacy of anti-cancer drugs in human tumors.

Human tumors will be selected according to the following criteria:

-frequently encountered
-responsive to chemotherapy
-patient with evaluable disease

These tumors will be transplanted in the nude mice system. The same chemotherapeutic regimen will be given both to the patients and to the mice. Correlation will be studied between animal and clinical response to chemotherapy.

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